Biologically Active Peptide Analogues

A thesis

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of

Doctor of Philosophy in Chemistry

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by

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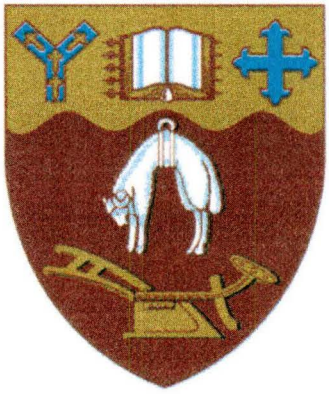

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1996

Acknowledgments

I have thoroughly enjoyed the time I've spent pursuing my research. This is a reflection of the encouragement and good humour of my supervisor Dr. Andrew Abell. I thank him for his support. I am also extremely grateful to my associate supervisors Dr. John Blunt and Dr. Murray Munro for their guidance and enthusiasm. Many other staff and students in the Department of Chemistry have contributed to my time at Canterbury University, with their advice and friendship.

I would like to dedicate this thesis to my parents Cilla and Maurice and my sister Nicky, for their fantastic support.

I feel fortunate to have good friends and family and I thank them for making everything worthwhile.

Contents

Abstract

Biologically active peptides and their receptors are involved in many of life's essential processes. They are of enormous medical interest as many diseases and illnesses can be treated by agents which block or imitate the function of specific peptides and their receptors.

The research described in this thesis encompasses two distinct approaches towards the development of peptidomimetics, analogues which mimic specific biologically active peptides. Chapter one gives a general introduction to the role of peptides in drug discovery and design and some representative examples.

Chapters two deals with the rational design of a new *cis*-hydroxyethylamine peptidomimetic isostere and its application to the development of HIV drugs. Chapter three describes the synthesis of 1,5-disubstituted tetrazole dipeptide compounds, based on the designed isostere, and their extension in the *N* and *C* directions. The HIV-1 protease inhibition results of a series of tetrazoles are presented in chapter four. Compounds based on the designed isostere gave reasonable μM Ki values, compared with the nM values of the most potent known HIV protease inhibitors. The hydroxymethylene group was found to be crucial to activity and the QC ligand was shown to be favoured at the P_3 site over Cbz. The C3 and C6 configurations adjacent to the tetrazole ring were also shown to influence the activity of the inhibitors. These design principles on which the *cis*-hydroxyethylamine isostere was based were justified and have given a basis for future development of the structure.

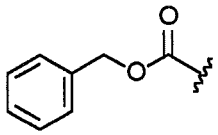
A more traditional approach to drug discovery is the exploitation and development of the biological activity of potent natural products. Previous work has established the potent activity of the α-hydroxyamidoacetal C7-C10 functionality of the sponge metabolites mycalamides A and B. Chapter five describes two synthetic routes to a general α-hydroxyamidoacetal structure (see **5.5**), and the synthesis of a series of mycalamide analogues with varying R_1-R_4 substituents, for example the epimers (*1'S,2S*)- and (*1'R,2S*)-2-hydroxy-*N*-(1-methoxybenzyl)-3-phenylpropanamide. The assignment of configuration and antitumour activity of the series of analogues is presented in chapter six. The compounds show modest *in vitro* antitumour activity.

The level of activity appears to be more sensitive to changes at R_2 than R_1 and to favour a (*1'R*)-configuration. The amino acid based analogues (R_1 = NHR) showed comparable or improved activity over the R_1 = OR analogues, and supported the proposal that the series of analogues act as peptidomimetics.

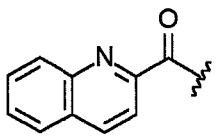
Chapter seven details the X-ray crystallographic analysis of a crystal of $(DMAP)_5(benzoic\ acid)_3(H_2O)_{10}$, with a unique layered structure in which benzoate anions are two-dimensionally clathrated by water molecules.

Abbreviations

ACE	angiotensin converting enzyme
AIDS	acquired immune deficiency syndrome
BOP	benzotriazolyltris(dimethylamino)phosphonium hexafluorophosphate
Cbz	benzyloxycarbonyl



DCC	dicyclohexylcarbodiimide
Diq	decahydroisoquinoline
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
GSH	glutathione
HIV	human immunodeficiency virus
HOBT	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
IC_{50}	concentration required to inhibit cell growth by 50%
NCI	National Cancer Institute
nOe	nuclear Overhauser effect
Pth	phthalyl
QC	2-quinolinylcarbonyl



TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMSCN	trimethylsilylcyanide

Chapter One

Introduction

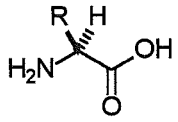
Peptides are essential to sustaining life in nature. In humans biologically active peptides are involved in vital processes such as blood pressure regulation, respiration, digestion, metabolism, reproduction, immune defence and sensitivity to pain. These processes involve the interactions of specific peptides, hormones, neurotransmitters and enzyme substrates, with receptors or enzymes. Thus, peptides are of enormous medical interest, being implicated in many diseases and illnesses and having applications in the development of therapeutic agents. Medical conditions including hypertension, emphysema, gastrointestinal diseases, diabetes, fertility disorders, Acquired Immuno Deficiency Syndrome (AIDS), prostate cancer, neuropsychiatric disorders, pain and inflammation can be treated with therapeutic agents which block or imitate the function of specific biologically active peptides and their receptors or enzymes.

What follows in this introduction is a brief discussion of the structure and properties of peptides and some representative examples. Finally, a discussion of the role of peptides in the process of drug discovery and design, with a particular emphasis on natural product-based systems and peptidomimetics.

Peptides and Proteins

Peptides and proteins (there is no clear boundary between these two classes of compounds) consist of varying sequences of amino acid building blocks joined by amide linkages. Despite an apparent lack of diversity, they serve a variety of purposes in living organisms: structurally important proteins such as keratin in skin and collagen in tendons; enzymes (large flexible, globular proteins which catalyze many important chemical reactions); and smaller peptides such as hormones (from the Greek for "to set in motion") and neurotransmitters which can act as chemical messengers. Proteins also function in the interconversion of chemical, mechanical and light energy forms.

Twenty primary amino acids exist in proteins in nature, with several hundred more non-protein amino acids having evolved from the diverse metabolism of species, mainly from the plant world and micro-organisms. Humans and all animals must obtain the essential primary amino acids in their diets from the plant kingdom. The biosynthesis of the primary amino acids in plants starts with ammonia, itself formed by the fixation of nitrogen gas from the air or the reduction of nitrate from the soil. In contrast to animal metabolism, plants conserve all the available nitrogen that they intake. The identification of the primary amino acids from various natural sources occurred over a century[1] from 1820, when Braconnet characterized glycine and leucine, until threonine was isolated in 1925. α-Amino acids contain an amino (NH_2) group, a carboxylic acid (COOH) and a sidechain group R attached to a central carbon (figure 1.1). The common amino acids differ only in the nature of the R sidechain.



L-α-amino acid

Figure 1.1: General structure of the primary amino acids.

Of the two possible enantiomers, only that designated as the *L*-enantiomer is found in most peptides and proteins. A variety of sidechains exist with different functional groups, structures and properties. Amino acids are linked together by condensing the amino group of one with the carboxylate of another to form an amide or peptide (CONH) bond. Two or more linked amino acids are called a peptide while a larger polypeptide may be termed a protein. An amide bond has partial double bond character due to conjugation of the lone pair of nitrogen electrons with the carbonyl double bond. Consequently, there is no free rotation in the peptide chain about the amide bonds, with two slowly interconverting, *cis* and *trans* coplanar forms possible (figure 1.2). The *trans* form is energetically favoured over the *cis* due to less steric hindrance. However, *cis* amide bonds can be found in peptides containing *N*-alkylated amino acids (such as proline), in cyclic peptides, and in peptide chain folding where they are more energetically accessible. The typical charge separation in amide bonds

means that the peptide backbone is quite polar and suitable for hydrogen bonding. The positively charged hydrogen on nitrogen readily interacts with negatively charged carbonyl oxygens (figure 1.2).

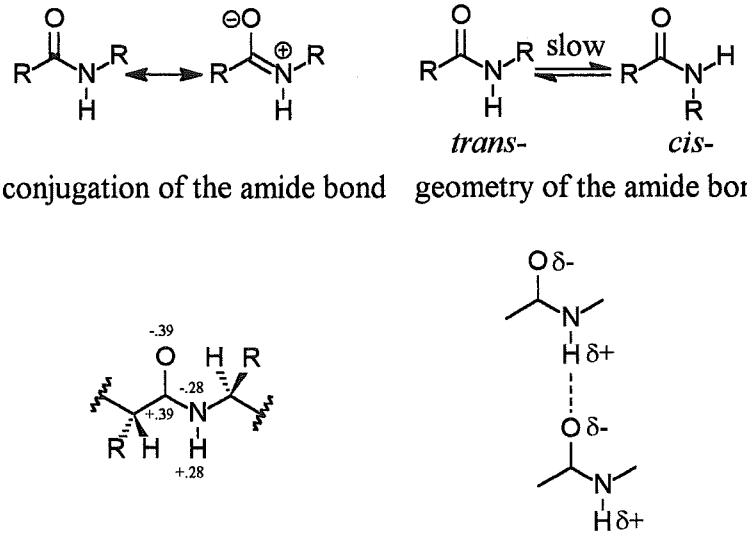


Figure 1.2: Properties of an amide bond.

Peptide chains are folded or coiled in an ordered way to give secondary and tertiary structure. Ordered three-dimensional structures such as α-helices and β-pleated sheets are exhibited with a high degree of regularity in proteins despite the huge number of possible conformations.[2] The biological properties of peptides and proteins vary greatly with sequence and ultimately three-dimensional structure. The prediction of three-dimensional protein structures from amino acid sequence, and the understanding of the relationships to biological function, has been the focus of much research.[3]

Peptides and proteins with useful biological properties and complex functions have evolved in nature by natural selection, and their blueprints encoded in the genetic material DNA. Protein synthesis occurs in cells on the ribosomes, under the direction of messenger RNA (mRNA) molecules, which transfer the protein codes from the DNA in the cell nucleus. The amino acids for protein synthesis are brought to the ribosomes by specific transfer RNA (tRNA) molecules.

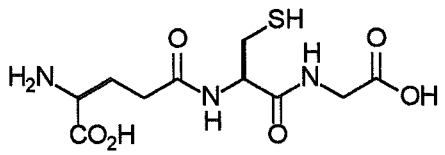
As advances in chemistry, medicine, and molecular biology are made, more biologically active peptides and proteins continue to be discovered and characterized, and their functions and interactions more clearly understood.

Free peptides in large concentrations are rare in humans. For peptide hormones to carry messages between remote cells it seems that a large amount of hormone would need to be produced. This is avoided by the extremely specific recognition of the hormone by receptors only at the target site. Other receptors along the way do not recognize and bind incompatible ligands. Peptides can also act as neurotransmitters. They are released at nerve endings and evoke a response after interacting with receptors on a nerve cell.

Proteases are an important class of enzyme which catalyze the hydrolysis of amide bonds in peptides. Their biological activities are closely interrelated with a number of peptides. Certain proteases can liberate peptide hormones and neuromodulators from inactive precursors, while others repress biological response by degrading these peptide messengers. They are strictly regulated by endogenous peptide inhibitors.

Enormous interest has developed in the discovery of naturally occurring peptides and the elucidation of their biological activities. What follows is a brief discussion of some biologically important peptides.

Glutathione (GSH)



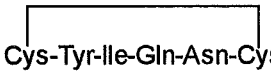
1.1

GSH is a tripeptide first observed as a reducing agent in yeast in 1888 and later identified as γ-*L*-glutamyl-*L*-cysteinyl-glycine. GSH is the most abundant intracellular thiol in almost all aerobic biological species due to the relative stability of its γ-peptide bond to degrading protease enzymes. The biochemical functions of GSH are mostly

due to the thiol functional group and include protecting cells against the toxic, radical and non-radical products of oxygen.

An interesting effect of GSH, not related to the thiol group but to the recognition of the peptide backbone,[4] is observed in hydra (small, freshwater hydrozoan polyps). A contraction of the polyps' stinging tentacles to trap its prey is biochemically induced by 10^{-6} M GSH.

Oxytocin and Vasopressin



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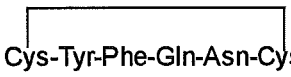

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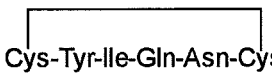


1.4

(oxytocin ring) (vasopressin sidechain)

The peptide hormones[5] oxytocin **1.2** and vasopressin **1.3** are released by the pituitary gland, an important regulatory gland found at the base of the brain. Oxytocin causes rhythmic contractions of the uterus to initiate childbirth and the release of milk from the mammary gland, whereas vasopressin raises blood pressure and has an antidiuretic effect. The discovery of such hormones at the turn of the century led to the realization that physiological responses can be initiated by biochemistry. All physiological responses were previously thought to be caused by electrical impulses through the nervous system.

A deficiency of vasopressin causes excessive excretion of water and leads to the unpleasant condition *diabetes insipidus*. Large scale synthesis of vasopressin[6] has allowed control of the disease in patients.

Extensive research on the structures of oxytocin and vasopressin led to great advances in peptide synthesis and elucidation, and a Nobel Prize for Vincent du Vigneaud.[7] Each structure contains an intramolecular disulphide bond and a glycinamide at the *C*-terminal, and only differ to each other by two amino acid

residues, despite their differences in activity. A number of receptors are involved in their biological activities. A conceivable common evolutionary ancestor of the two principle pituitary hormones, vasotocin **1.4**[8] was synthesized and later found in extracts of the pituitary glands of birds and reptiles, plus in the pineal gland of mammals. It contains the ring of oxytocin and side chain of vasopressin, and exhibits the biological activity of both.

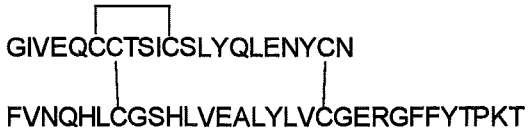
Renin-Angiotensin System

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe **1.5**

The renin-angiotensin system plays an important role in the regulation of blood pressure.[9] An increase in cases of cardiovascular diseases in humans, such as hypertension and heart failure (responsible for 10% of deaths in western society[9]), lead to enormous interest in this system.

The system produces angiotensin II **1.5**, one of the most potent known vasoconstrictors. It starts with the protein angiotensinogen (mass of about 60kDa) which is synthesized in the liver and passes into the blood. Angiotensinogen travels to the kidneys where it is cleaved by the aspartic protease renin to give angiotensin I. Angiotensin I is then cleaved to the biologically active **1.5** by angiotensin converting enzyme (ACE), found primarily around the lungs. Release of **1.5** affects blood pressure directly by constriction of the blood vessels (after binding to specific receptors on the surface of the cells) and indirectly by release of the hormone aldosterone from the adrenal gland (inducing sodium ion and water retention). Inactivation of **1.5** occurs by enzymatic cleavage of the *C*-terminal residue to form angiotensin III. Successful antihypertensive agents[10] are being used in medicine which inhibit the action of the ACE enzyme on its peptide substrate angiotensin I. Agents which inhibit the action of renin on angiotensinogen have also been extensively studied however none have been successful in clinical trials.[10,11]

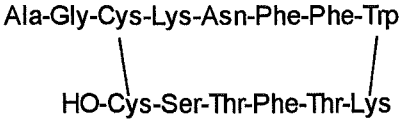
Insulin[12]



1.6

Insulin **1.6** is the primary peptide hormone responsible for glucose metabolism. It is released by specialized cells in the pancreas and acts on nearby liver cells to suppress the breakdown of glycogen to glucose. A deficiency of **1.6** causes the disease *diabetes mellitus* which affects millions of people around the world. Treatment of patients is possible through industrial synthesis of human insulin by biogenetic engineering. This involves harnessing the protein synthesizing machinery of microorganisms with the appropriate DNA sequences incorporated into the cells. Insulin has a complex three-dimensional structure consisting of two peptide strands joined by disulphide linkages.

Somatostatin



1.7

Somatostatin[13] **1.7** is a cyclic peptide formed in the hypothalamus. Its biological activity is as a release-inhibiting factor, preventing the release of growth hormone (GH) from the pituitary gland. It also acts on the pancreas, preventing the release of both insulin and glucagon, leading to a lowering of blood glucose concentration and is thus interesting in developing possible treatments for *diabetes mellitus*. In the digestive tract, motility is inhibited by somatostatin, the blood supply restricted and secretion of gastric acid and gastrin reduced. These properties have led to therapeutic use of somatostatin in the treatment of acute gastrointestinal diseases. Receptors for somatostatin have been isolated and biochemically characterized.

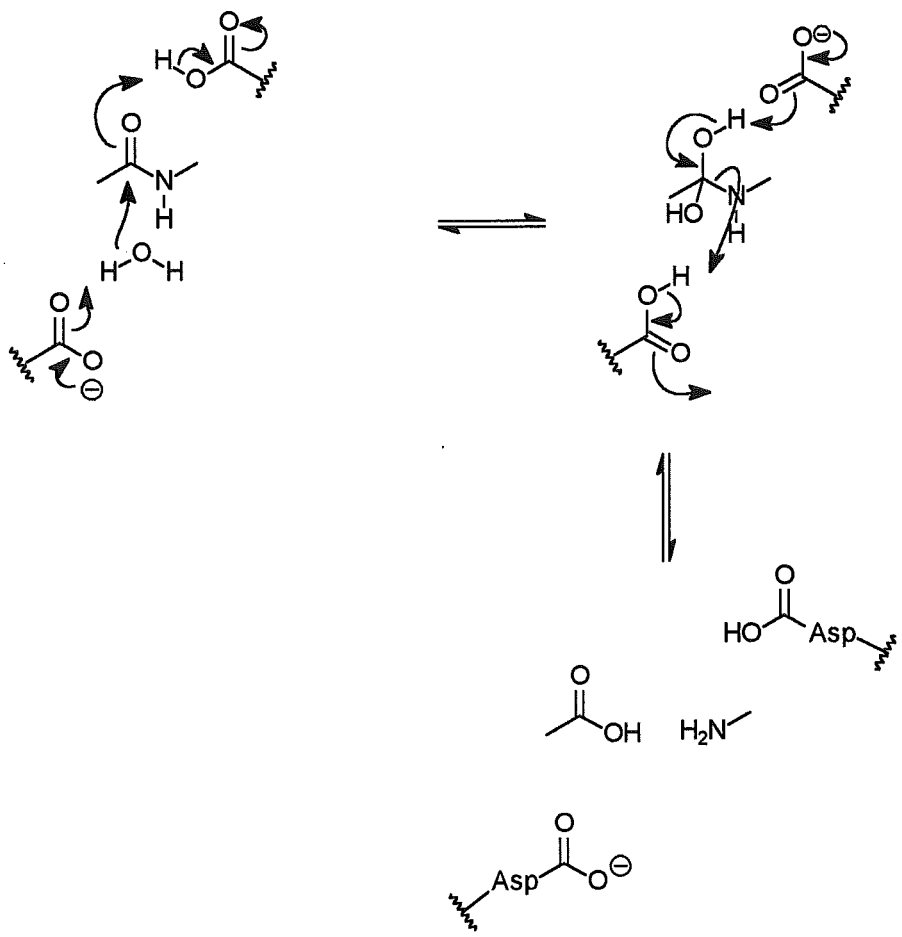
Enzymes and Receptors[14]

Enzymes are proteins which act specifically on peptide or non-peptide substrates catalyzing many chemical transformations. Almost every biological bond-breaking and bond-forming reaction, including the breakdown of food and its reconstruction into other biomolecules, is catalyzed by one or more enzymes.

Enzymes are characterized by the chemical reactions they catalyze and their substrate specificity. Proteases are an important class of enzyme which catalyze the hydrolysis of amide bonds in peptides and proteins. This class of enzyme is the topic of some of the research presented in this thesis. Proteases are divided into types (four common types are shown in table 1.1) according to their mechanism of action (an example is the aspartic protease mechanism, scheme 1.1).

Table 1.1: Types of proteases.

Protease type	Significant Active site groups	Examples	Normal Function
Aspartic	Asp	renin	blood pressure regulation
		HIV protease	HIV replication
		pepsin	digestion
Serine	Ser His Asp	chymotrypsin	digestion
		thrombin	blood coagulation
		post protein cleaving enzyme	hormone metabolism
Metallo	zinc ion	angiotensin converting enzyme	blood pressure regulation
		carboxypeptidase	digestion
Cysteine	Cys	cathepsins B, H, L, calcium activated neutral proteases	protein turnover, bone resorption



Scheme 1.1: Aspartic protease catalyzed hydrolysis of an amide bond.

All enzymes possess an active site cavity where catalysis takes place. The active site is also sensitive to chirality, meaning most reactions are stereospecific. Proteases bind very specifically to peptides. Peptide sequence and conformation is crucial to favourable enzyme-substrate interactions. A protease active site contains pockets, into which sidechain groups of specific amino acid residues can project, and a scaffolding of potential binding points (through hydrogen bonding, hydrophobic and electrostatic interactions). The active site of a protease to which a peptide substrate binds is defined in terms of a series of subsites (figure 1.3).[15] Amino acid residues of the substrate are sequentially designated P_1, P_2, P_3...P_n and P_1', P_2', P_3'...P_n' in the *N*-terminal and *C*-terminal directions, respectively, from the amide cleavage site P_1-P_1'. These residues of the substrate are located in subsites of the enzyme designated as S_1,

S_2, $S_3 \ldots S_n$ and S_1', S_2', $S_3' \ldots S_n'$, respectively. The specificity pockets/subsites of the enzyme determine the amino acid residues accepted by the active site and define the bioactive conformation.

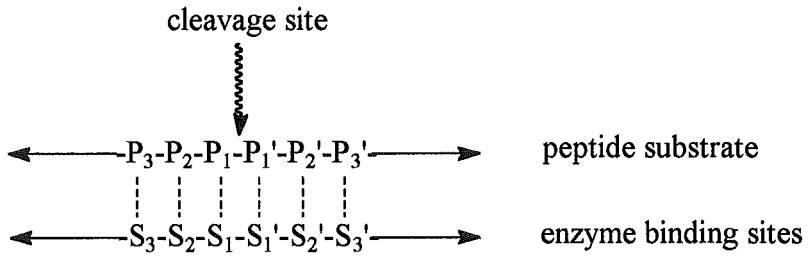


Figure 1.3: Schechter[15] notation for protease-substrate binding.

As the substrate binds in the active site, there is a structural change in the overall protease-substrate complex which brings a suitable catalytic group (an example is the aspartate residues in scheme 1.1) of the enzyme to bear on the substrate. The amide bond is cleaved and the products dissociate from the active site. Enzymes bind most tightly to the transition state of the reaction they catalyze, stabilizing it and lowering its free energy. This means that the activation energy is lowered relative to the equivalent uncatalyzed reaction, and rates of reaction can be greater than 10^{12} times faster.

The stereospecificity of the active site has great possibilities for the use of enzyme catalysis in organic synthesis and has recently been reviewed.[16]

Many other receptors exist which do not participate in chemical reactions like enzymes, but do participate in the transfer of information and signals between cells, by the recognition of active compounds such as hormones and neurotransmitters. These receptors are also proteins and structurally similar to enzymes. Receptors are characterized by the nature of their ligand. Receptors are usually located on cell membranes and the binding of an active compound occurs as for enzyme-substrate complexes, where a structural change takes place. As a result of binding, a characteristic effect occurs. The function of receptors is to receive and filter signals and to transmit the correct information on to another site where, for example, an

enzymatic reaction might be affected. The insulin hormone receptor is an integral membrane glucoprotein which binds insulin **1.6**, migrates and makes the hormone available to be metabolized within the cell.

Drug discovery and design

There are two basic approaches to drug discovery, rational design and mass screening. The expanding knowledge of important peptide-receptor complexes and their specific function has lead to a more rational approach in the design of therapeutic agents (from this point on, the term drug receptor will be used in a generic sense to encompass both enzymes and receptors, as discussed above). Computer assisted molecular modelling, X-ray crystallographic analysis and NMR spectroscopic methods have been instrumental in the elucidation of important biological processes. The shift in philosophy towards rational design should continue until successful drugs are able to be designed *de novo* with predictable biological activity. At present mass screening programs for lead ligands is a valuable process to give starting points in drug development.

A major goal in medicinal chemistry is the identification of potent and specific ligands to biological receptors. Identification of such ligands is a fundamental step in probing ligand-receptor function and developing therapeutic agents. Advances in genetic engineering in the 1970s lead to the availability of large quantities of many biologically important peptides and proteins. As a result the identification of target receptors has accelerated and demands on screening for ligands has greatly increased. Random and mass screening of natural products and existing compound databases are extremely labour and time intensive. Therefore recently, enormous efforts have gone into the synthesis of combinatorial libraries where in excess of 50,000 different compounds can be synthesized rapidly and screened for biological activity.

Combinatorial chemistry has been described as the science of efficient divergent synthesis.[17] Compounds based on common building blocks such as peptides and oligonucleotides are ideal to form such libraries as a vast number of combinations are possible. If tetrapeptides based on combinations of the twenty primary amino acids

were synthesized then 160,000 (20^4) products would be possible. The libraries can be based on a random strategy of screening every possible combination of building block, or a directed strategy of screening mutations of a lead compound.

The preparation of such combinatorial libraries[18] can be achieved by the split synthesis technique on small chemically inert polymer beads. For example, you can take 100,000 polymer beads (10 μm diameter) and attach the same starting molecule to each. Then divide the beads into five groups and perform a different chemical reaction on each. Mixing of the beads, dividing into five new groups and performing another five reactions generates 25 compounds. Repeat this process four more times and a staggering 15,625 different compounds are generated, each attached to its own bead. An essential step is to tag the molecules as they grow, so that each can be identified at the end. Several techniques are available for tagging, including adding DNA nucleotides at each stage to a different site on the bead. The DNA sequence can then later be identified by using the polymerase chain reaction. A recent development is tagging the molecules using microchip memories. Beads containing microchips can be sent radio signals at each stage to record details, to be read in an instant when synthesis is complete.

Fast and effective screening of combinatorial libraries for desirable biological activity is required to prevent a bottleneck developing, with the enormous numbers of different compounds being generated. One potentially powerful technique is the use of an appropriate antibody which will bind to desirable ligands. By linking the antibody to an enzyme that causes staining when the antibody binds, the desirable molecule in the library can be identified.

Many new building blocks and reactions from organic synthesis are being applied to the preparation of more rational small molecule libraries.[19]

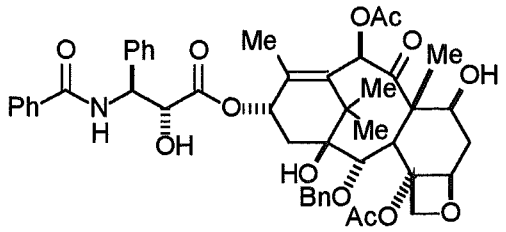
Nature provides a wealth of interesting compounds (peptidic and non-peptidic) with novel structures and potent biological activities, which provide previously undreamt of new ideas and leads in the development of therapeutic drugs.

Traditionally, a random and mass screening of natural products, followed by synthetic manipulation and refinement, has been employed to discover lead compounds in drug development. It is not unusual for natural products from one organism to bind to receptors in another.

Throughout history humans have been interested in naturally occurring compounds. Natural products were once the only source of medicines for mankind. Healing creams and liniments were produced from plant extracts in practically all ancient cultures. Even chimpanzees have been known to chew certain leaves only when suffering from gastro-intestinal problems. Natural products have been used in medicine since ancient times. In China, the *Pen-Tsao* was compiled by Shen Nung around 2800 BC and describes 365 herbal drugs used in those times. One of the first known drugs ephedra, described by Shen Nung and also included in ancient Greek medicine, was isolated from the 'horsetail' plant *ma huang*. It has been used as a stimulant to remedy respiratory diseases, to induce fever and perspiration, and to depress coughing. In the seventeenth century, the Jesuits brought with them from South America some medicinal concoctions developed by the Incan Indians, including the bark of the china tree for the treatment of malaria. In 1820 Pelletier and Caventou isolated the active component, quinine, from the china tree and sparked a rapidly growing interest in isolating medicinal natural products.

More recently the development of modern chromatographic techniques has enabled the separation of complicated mixtures of compounds from natural extracts. The process of purification can be guided by bioassays indicating which components of the mixtures have useful biological properties. Natural products being screened for drug discovery are isolated from many different sources. The National Cancer Institute (NCI) organizes enormous screening projects including compounds extracted from all three major organism types: plants, animals and microbes. The marine environment is a more recent source of potentially useful natural products. The Roche Institute among others are involved in major efforts to collect a wide variety of organisms from the world's oceans. What follows is a brief discussion of some important natural product pharmaceuticals.

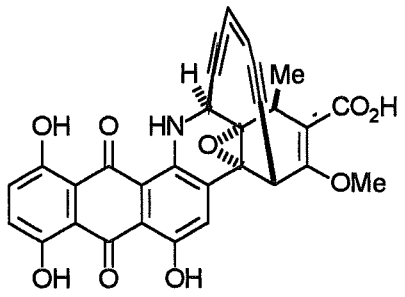
Paclitaxel



1.8

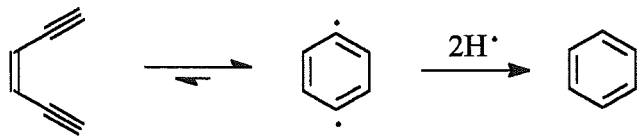
An extensive screening of plant materials, as part of the NCI campaign against cancer, lead to the discovery[20] of paclitaxel **1.8** from the bark of the Pacific Yew tree. It has been hailed as a miracle drug against cancer but is in short supply due to its natural scarcity. To obtain one kilogram of paclitaxel, 3000 rare and slow-growing trees would have to be sacrificed. Ironically, the yew tree was regarded as the tree of death by the Greeks, because of its poisonous extracts which were commonly used in suicide and murder attempts. Paclitaxel has a unique mechanism of action,[21] causing cancer cell death by stabilization of microtubules (large proteins involved in many aspects of cellular biology). The novel molecular architecture of **1.8** was determined by X-ray crystallography and has recently been the subject of enormous synthetic efforts. The Nicolaou[22a] and Halton groups[22b] simultaneously reported the challenging first total syntheses of **1.8** after a heated race with thirty or so groups around the world, however these syntheses are unsuitable as large scale sources of **1.8** for drug purposes. Semisynthetic supplies[23] of **1.8** are now available which should meet the demands of ongoing clinical trials.

Enediynes



1.9

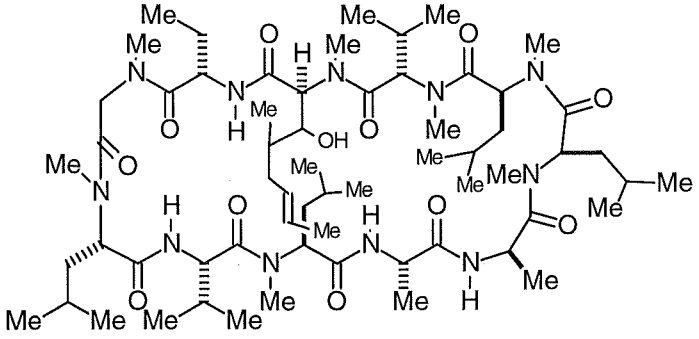
A new class of natural products, the enediyne anticancer antibiotics[24] (an example is dynemicin A **1.9**), were discovered from various bacterial sources and exhibit potent anticancer activities. They possess novel molecular architecture, potent biological activity and fascinating mechanisms of action. Their activity is exerted by the enediyne ring which undergoes Bergman cyclization[25] (scheme 1.2) to form a 1,4-benzenoid diradical species. This species is very damaging and leads to cleavage of DNA strands. This intriguing cycloaromatization was demonstrated separately in 1972, however its full biological significance only became apparent after the enediynes were revealed by nature. Potentially selective activity of certain enediynes for cancer cells can stem from their ingenious triggering and delivery systems, which means cycloaromatization of the enediyne does not occur until the molecule has reached its target. This class of natural product has sparked the development of a number of potent and therapeutically useful analogues. Selected enediynes are in clinical trials.



enediyne

Scheme 1.2: Bergman cyclization.[25]

Cyclosporin A and FK506



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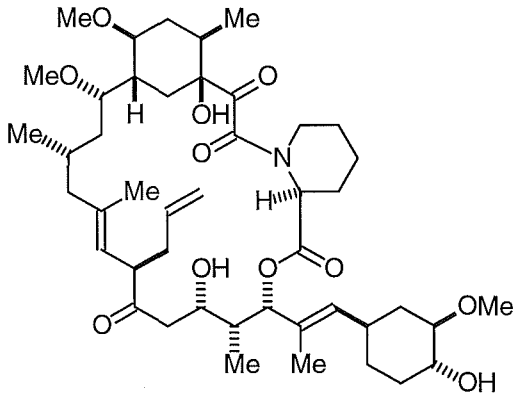


1.11

Cyclosporin A **1.10**[26] and FK506 **1.11**[27] are fungal natural products that inhibit the specific signal transduction pathways which lead to T-lymphocyte activation. They are employed as immunosuppressive agents to prevent graft rejection after organ and bone marrow transplantation, and are responsible for a revolution in clinical transplantation. Cyclosporin A binds to a protein receptor called cyclophilin that is identical to the enzyme peptidyl-prolyl isomerase[28] which catalyses the interconversion of *cis* and *trans* rotomers of peptidyl-prolyl amide bonds in peptide substrates. FK506 binds to a similar receptor FKBP. Research on the biological activity of these immunosuppressants is unravelling the complex cascade of events leading to T-cell activation.[29]

Peptidomimetics[30]

Peptides are a rational starting point in drug design due to their biological significance. They can have potentially high specificity and affinity for a target receptor, as discussed for substrate-enzyme complexes above. Unfortunately these factors are often negated by their poor pharmacological properties of low metabolic stability and poor absorption after oral ingestion. Peptides are susceptible to hydrolysis by proteases in the gastrointestinal tract, inside cells, and in serum, and are rapidly excreted through the liver and kidneys. Due to their relatively high molecular mass and lack of specific transport systems, they are not absorbed readily after oral ingestion.

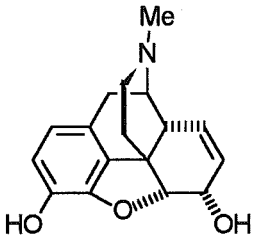
So although a peptide may have potent activity against a target *in vitro*, it may not have high enough concentration at the target site *in vivo* to be an effective drug. Peptides have been employed as drugs in only a few cases: insulin **1.5** and vasopressin **1.3** are given to patients suffering from the deficiency diseases *diabetes mellitus* and *diabetes insipidus*, respectively.

Therefore, a logical step in rational drug design is to convert the three-dimensional structural information contained in biologically active peptides to small non-peptide ligands. Such compounds, called peptidomimetics, are expected to have more favourable pharmacological properties and still retain the potency and selectivity of parent peptides.

Peptidomimetics are based on knowledge of the conformational, topochemical and electronic properties of a native peptide and its receptor.

A most important principle in the design of peptidomimetics is the formation of conformationally restricted ligands which mimic the bioactive conformation of the native peptide. If necessary the required bioactive conformation can be stabilized by introducing elements which force rigidity. Structural elements are added to a ligand in defined positions which ensures that important hydrogen bonding, electrostatic and hydrophobic interactions can still occur with the receptor. Mass screening of natural products and combinatorial libraries (discussed above) produce lead compounds which are often conformationally restricted analogues of peptides that have the required properties to be recognized by the receptor or enzyme. An example is morphine, an

extract of the opium poppy, which has been used and misused for its pain-killing and euphoria-generating effects. Morphine **1.12** mimics the three-dimensional structure of endogenous peptides called enkephalins (figure 1.4), at a common receptor.[31] Enkephalins are rapidly degraded in the body and so are poor pain-killing drugs. However, elucidation of their structure-activity relationships has resulted in useful synthetic analgesics more potent than morphine.



1.12

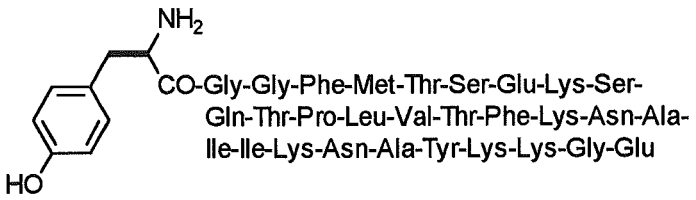
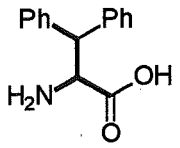


Figure 1.4: Morphine **1.12** imitating β-endorphin

Several strategies have been established for the design of conformationally restricted and metabolically stable analogues of parent peptides of interest, and some of these are detailed below.

Side chain modification of amino acid residues



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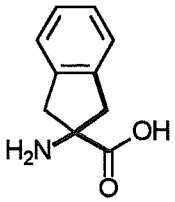
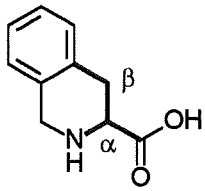
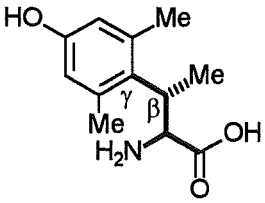


1.14



1.15



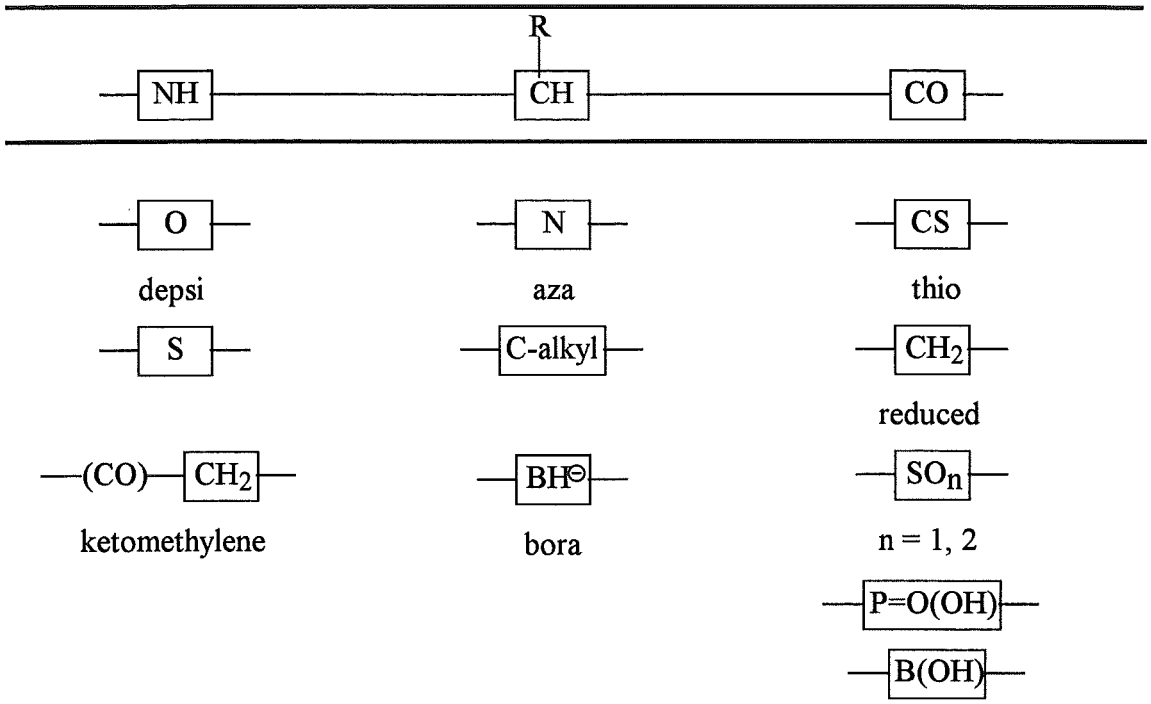
1.16

A well established strategy is the replacement of natural amino acid sidechains with unnatural derivatives. Conformational restriction is possible by introducing sterically demanding groups or rigid bridging units. Phenylalanine analogues **1.13** and **1.14** have been employed in potent ligands of the angiotensin II receptor.[32] Compound **1.15** is a phenylalanine analogue where the dihedral angle C^{α}-C^{β} is restricted to a very narrow range. It has been incorporated into various opioid antagonists with high selectivity for the μ receptor.[33] Another example **1.16**, involves modification of the tyrosine side chain by introducing methyl groups at the 2′, 6′ and β positions which hinders free rotation about the C^{β}-C^{γ} bond and can favour the formation of bioactive conformations.[34]

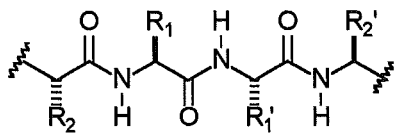
Modification of the Peptide Backbone

Isosteric or isoelectronic replacement of units in the peptide backbone can lower the peptide character of the molecule and increase its biological half-life, while still retaining favourable properties of the parent peptide. The most general replacements are shown in table 1.2.

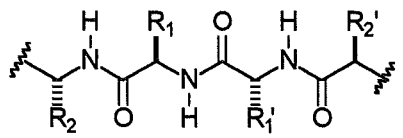
Table 1.2: General modifications of the peptide backbone.



Another approach is retro-inverso modifications (figure 1.5)[35] where natural *L*-amino acids are replaced with their *D*-enantiomers and the direction of the peptide chain is reversed. The small number of retro-inverso derivatives[36] that show activity comparable to their parent peptides indicates the importance of the peptide backbone for recognition by the receptor.



natural peptide



retro-inverso modification

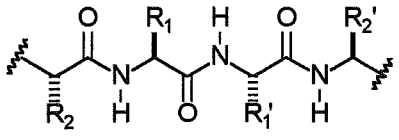
Figure 1.5: Retro-inverso modifications of a natural peptide

Replacement of an amide bond can make the molecule more hydrolytically stable to protease enzymes, force bioactive conformations and mimic reaction pathways. Various amide bond replacements are discussed below.

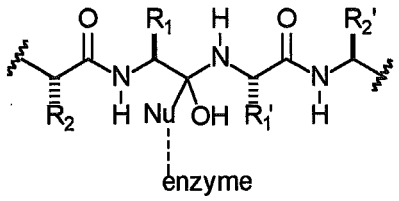
Dipeptide analogues

A classical approach in the design of enzyme inhibitors is the incorporation of hydrolytically stable dipeptide isosteres into peptide sequences. This approach was developed after the discovery of a variety of novel enzyme inhibitors from screening done on culture filtrates of microorganisms.[37] The peptide natural product pepstatin **1.17** was discovered to be an inhibitor of aspartic proteases (pepsin, renin, cathepsin D, zymosin and later HIV protease) and contained a previously unknown γ-amino acid statine. Statine mimics the tetrahedral intermediate formed by enzyme-catalyzed hydrolysis of an amide bond (figure 1.6). A ligand which more closely resembles a transition state or intermediate species of a reaction would be expected to bind better to the catalyzing enzyme, than one resembling the starting substrate. X-ray crystallographic studies of enzyme-inhibitor complexes have confirmed that the (*3S*)-hydroxyl group of statine hydrogen bonds to catalytically essential aspartate residues in the active site.[38] These clues on reaction mimetics provided by **1.17** lead to the idea of transplanting critical features of active natural products into other structural frameworks to develop selective and potent inhibitors of different protease enzymes.

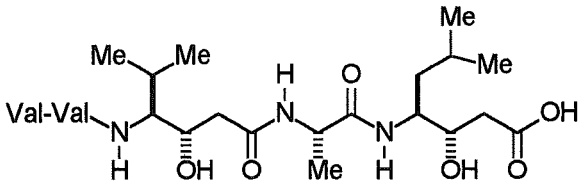
Statine as a γ-amino acid is one atom short of being a true dipeptide mimic based on the natural substrate, and so a series of related but previously unknown structures were designed (figure 1.6). Typically, these dipeptide isosteres incorporate a hydroxymethylene transition state mimic and a replacement for the amide bond which is cleaved in the substrate. The isosteres also permit the selection of suitable sidechains for a target enzyme based on the sequence specificity of the substrate.



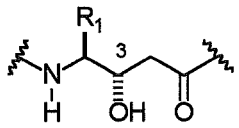
natural peptide



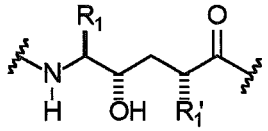
tetrahedral transition state
of enzyme-catalyzed hydrolysis



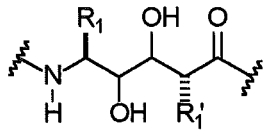
pepstatin **1.17**



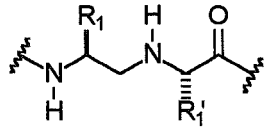
statine



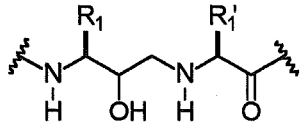
hydroxyethylene



dihydroxyethylene



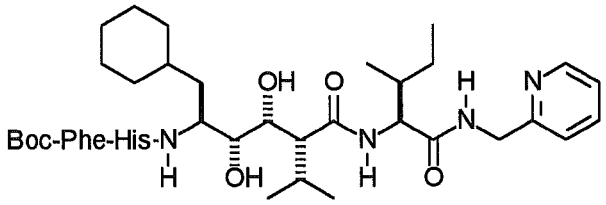
reduced amide



hydroxyethylamine

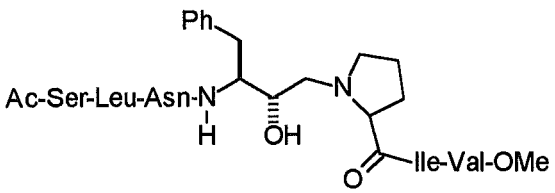
Figure 1.6: Hydrolytically stable dipeptide isosteres.

The hydroxyethylene isostere was the first of such structures used, being incorporated into the amino acid sequence of the renin substrate to produce potent renin inhibitors.[39] Renin was the subject of much interest due to its implications in cardiovascular disease. Molecular modelling studies suggested that the related dihydroxyethylene isostere might interact via both hydroxyl groups with the catalytic aspartate residues, and so compound **1.18** was prepared[40] and found to be a potent inhibitor of renin (IC_{50} 0.35 nM).

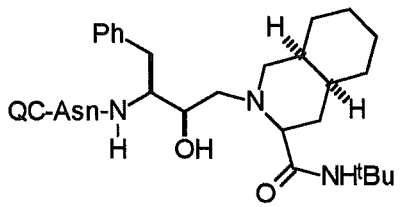


1.18

The hydroxyethylamine isostere has been incorporated into effective inhibitors of HIV protease[41] that mimic the Phe-Pro cleavage site in the viral protein *gag-pol*. JG-365 **1.19** was an initial potent inhibitor (IC_{50} 0.24 nM) based on incorporation of the hydroxyethylamine isostere into the minimum substrate peptide sequence required for activity. Extensive structure-activity relationship studies lead to inhibitor **1.20** (Ro 31-8959, IC_{50} 0.4 nM) with improved pharmacological properties. X-ray crystallographic studies of these HIV protease-inhibitor complexes has facilitated the development of new ligands.



1.19



1.20

HIV protease crystallizes as a C_2 symmetric homodimer with each amino acid chain contributing one catalytically essential aspartate residue. Pseudo-C_2 symmetric

isosteres were designed, based on this principle, and incorporated into potent inhibitors (figure 1.7).[42] Interestingly, the configuration of the COH centre has little effect on the activity of these compounds.

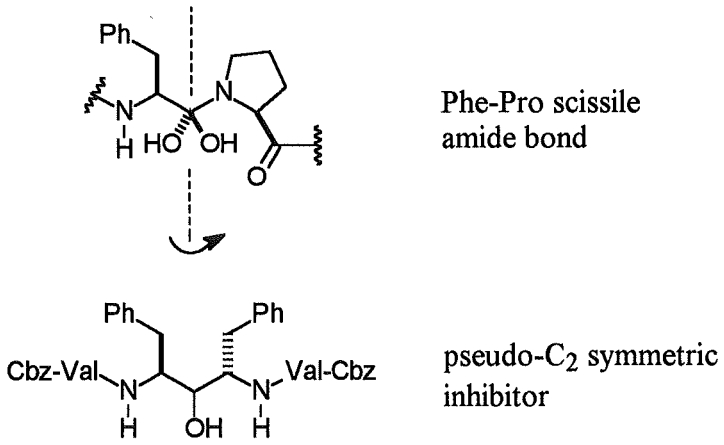
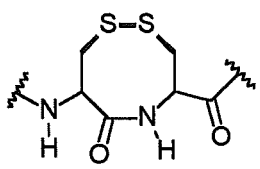


Figure 1.7: Design of a pseudo-C_2 symmetric HIV protease inhibitor.

Conformational Restriction

Conformational restriction[43] is an important principle in peptidomimetics. Analogues which assume the bioactive conformation of a target peptide are potentially more selective and potent ligands for a receptor. Also, ligands which are 'preorganized' for receptor-binding have a lower relative entropy cost. Techniques of conformational restriction include a variety of short or long-range cyclizations and the introduction of other rigid elements which make the molecule less flexible and may induce a favourable bioactive conformation. An understanding of the conformational implications of such restrained analogues is important to further design and development.



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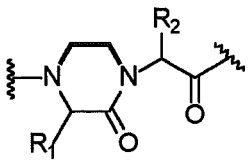

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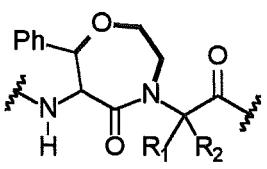
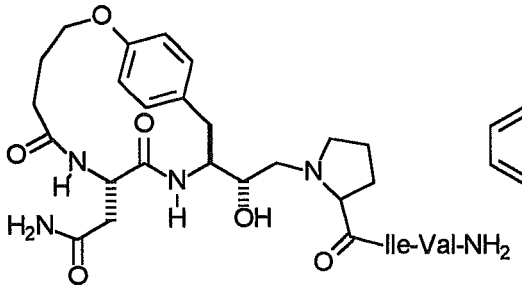


1.23

Substrate-based cyclization techniques can link two side chains **1.21**[44], two backbone units **1.22**[45] or a side chain and a backbone unit **1.23**[46]. Much research has been done on the synthetic problems encountered in developing such systems.

Many potent macrocyclic peptides are found in nature, such as somatostatin **1.7** and cyclosporin A **1.10**. The structure of cyclosporin A incorporates a number of peptidomimetic design techniques. It is cyclic, and possesses seven *N*-methylated amide bonds, several *D*-amino acids and some unusual *C*-alkylated substituents, which combined with intramolecular hydrogen bonding, all limit conformational freedom. In addition to their conformational restriction, macrocyclic peptides frequently possess more favourable pharmacological properties than linear peptides. Hydrophobic side chains of cyclic peptides provide a hydrophobic exocyclic surface that shelters cleavable amide bonds from degrading proteases and facilitates the penetration of cell membranes.



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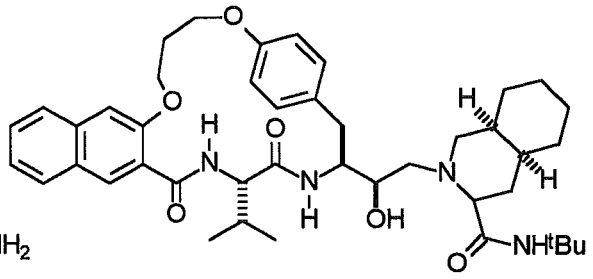
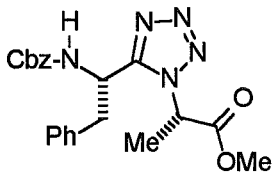


1.25

Synthetic macrocyclic peptidomimetics (for a recent review see Fairlie *et al.*[47]) have been developed based on X-ray crystal structures of HIV protease-inhibitor complexes. Macrocycles were incorporated into inhibitors **1.19** and **1.20**, to link the P_1 and P_3 residues which are arranged closely in space in the active site, to give compounds **1.24**[48a] and **1.25.**[48b] Molecular modelling of enzyme-bound structures superimposed well onto the X-ray crystal structure of bound **1.19**. Compounds **1.24**, **1.25** and derivatives are potent inhibitors (1-30 nM) of HIV protease and display enhanced metabolic stability.

Cis amide bond analogues

The partial double bond character of the amide bond leads to *cis* and *trans* isomers. In peptides the more stable *trans* configuration predominates. Exceptions include *N*-alkylated amino acids, cyclic peptides and turns (discussed below). Many *cis* amide bonds occur in the biologically active conformations of peptides. The biological activity of angiotensin II **1.5** is correlated with the isomerization of the His-Pro amide bond to the *cis* configuration.[49] The simulation of *cis* amide bonds which could occur in the biologically active conformation of a peptide is an important technique in designing peptidomimetics. Compounds **1.26**,[50] **1.27** and **1.28**[51] are examples of dipeptide analogues mimicking *cis* amide bonds. Each of these compounds incorporates a rigid ring structure as surrogate for the amide bond which restricts the relevant torsion angle to a value close to 0°. Incorporation of **1.27** into a cyclic hexapeptide analogue of somatostatin gave a compound with some biological activity.[52]



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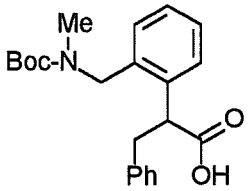

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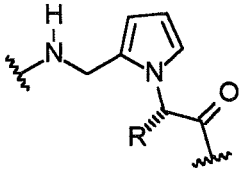
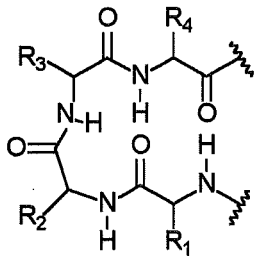
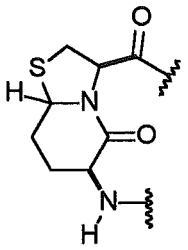


1.28

Imitation of secondary structure



β-turn



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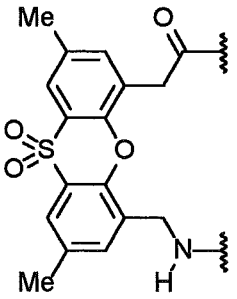
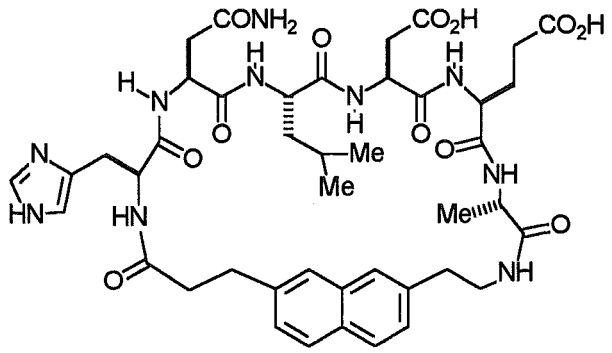


1.30

Figure 1.8: β-turn mimietics.

The retention of peptide secondary structure in mimetics is crucial to retention of biological activity. α-Helix and β-sheet structures, turns and loops are essential components of peptide and protein conformation. The β-turn (figure 1.8) is a structure common to many biologically active cyclic peptides, and has been postulated in many cases for the active conformation of linear peptides. In many proteins, β-turn structures are exposed and may be a part of ligand recognition sites. The secondary structures of peptides can interchange because of the inherent flexibility of peptides. Therefore, conformational restriction of bioactive secondary structures has been of great interest. The β-turn is the most frequently imitated secondary structure. Examples of β-turn mimetics include structures with still recognizable peptide chains **1.29**,[53] to those with completely non-peptidic components **1.30**. Structure **1.30** has been used to produce two antiparallel peptide chains[54] or, simply by changing the anchor groups, two parallel peptide chains (β-sheet mimetics).[55]

A mimic of Ω-loop secondary structures **1.31** has also been recently described.[56] A Ω-loop is a larger conformationally stabilized curve in a peptide chain, involving from six to sixteen residues. Such loops are found in many proteins and thought to have an important role in biological recognition. Structure **1.31** mimics the peptide sequence of residues 41-48 in interleukin-1α, an important mediator in immunological and inflammatory processes.[57]

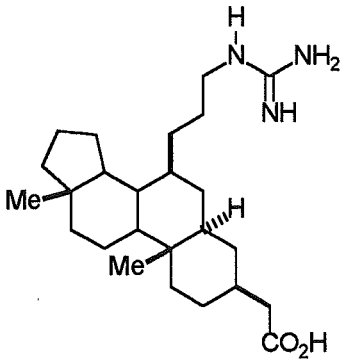


1.31

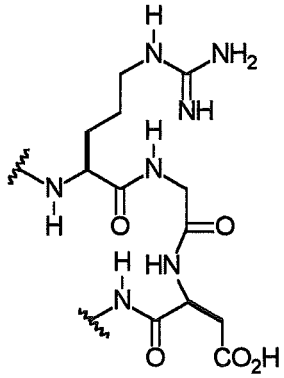
In addition, many of the techniques discussed above which restrict the conformational flexibility of a backbone and sidechains, can be applied to stabilize secondary structures. For example, the incorporation of *cis* amide surrogates into a backbone can stabilize the conformation of a β-turn. The use of such constrained units has been reviewed.[43]

Scaffold peptidomimetics

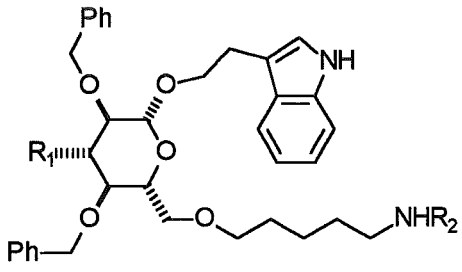
The support of essential side-chain elements on a completely unnatural framework is an interesting technique in designing compounds with favourable pharmacological properties. The structural frameworks are based on natural products or known drugs whose pharmacological properties are known. The relative conformational rigidity of such systems is important in mimicking the active conformation of a peptide. Also, the framework prevents hydrophobic collapse of the structure to an inactive conformation. Examples of scaffold mimetics and the original peptides are shown in figure 1.9. The steroid scaffold is a completely non-peptidic framework which is able to bear the appropriate sidechain groups to mimic a β-turn region of the bioactive peptide RGD.[58] β-*D*-Glucose is able to serve as the scaffold for mimics of a cyclic hexapeptide, which is known to bind to the somatostatin receptor.[59]



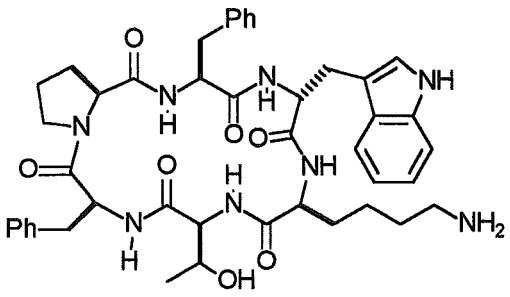
steroid scaffold



β-turn



glucose scaffold



somatostatin analogue

Figure 1.9: Examples of scaffold mimetics (left) and the original peptides (right).

Work Described in this Thesis

The research described in this thesis is divided into two related projects, which represent two distinct approaches towards the development of peptidomimetics as discussed above, rational design and the development of leads from natural products. Chapters two through four describe the design, synthesis and biological activity of a new, generally applicable *cis*-hydroxyethylamine dipeptide isostere, and its applications to the inhibition of HIV protease. The principles on which this rationally designed peptidomimetic is based are discussed above. It is hoped that the shift towards rational design will continue so that drugs may be designed *de novo*, based on a knowledge and understanding of receptor-ligand interactions.

Chapters five and six describe the synthesis and biological activity of a series of analogues, of a class of natural products with potent antiviral and antitumour activity. It is thought that the parent natural products exert their bioactivity through an inhibition of protein synthesis, and are therefore acting as peptidomimetics. As a result of previous, extensive, structure to activity correlations on the parent compounds mycalamides A and B, a minimum essential substructure required for retention of biological activity was proposed. The series of analogues described in this thesis was based on this substructure. This approach to drug development looks to exploit the biological activity of natural products, which can lead to highly selective, more potent and less toxic compounds.

Chapter Two

Design of a *cis*-Hydroxyethylamine Isostere

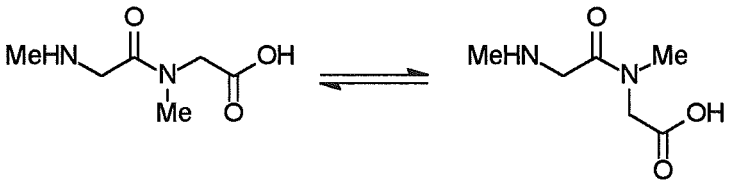
Introduction

A central underlying principle of peptidomimetics is conformational restriction (see chapter one for a discussion), where the flexibility of a system is restricted and bioactive conformations are promoted. The replacement of components of a target peptide with various structural modifications (isosteres) can be a probe of the conformational requirements for biological activity, by allowing different degrees of rotational freedom along the peptide backbone. Such modifications can also improve the pharmacological properties of the analogue by reducing its peptidic character, making it more biostable and hence more suitable as a therapeutic agent. The ultimate goal being to produce a non-peptidic ligand constrained to the appropriate bioactive conformation.

Cis-trans isomerization of amide bonds occurs as a result of the partial double bond character of the CO-NH bond. The configurations of the amide bonds in a peptide is of crucial importance to the conformation of the molecule and therefore, to its ability to bind to a receptor. In many cases, binding to a receptor will only occur with a *cis* configuration at certain amide bonds. *Cis* amide bonds can also occur in *N*-alkylated amides, secondary structures such as turns, constrained cyclic peptides and bioactive conformations.

In a normal amide bond the *trans* configuration is energetically favoured over the *cis* by approximately 10 kcal/mol, due to less steric hindrance between adjacent groups. In *N*-alkylated amides however, this energy difference is smaller (approximately 2 kcal/mol), due to both isomers having similar 1,4-steric interactions, and *cis-trans* isomerization can be observed by NMR. *N*-Alkylated amides are found in a variety of biologically important peptides and make the *cis*-amide isomer more energetically accessible for recognition and binding by the receptor. Proline is the only naturally occurring primary amino acid that leads to *N*-alkylated amides. Over 10% of proline

residues in protein X-ray crystal structures have been found with *cis* amide bonds. The NMR studies of morphiceptin (Tyr-Pro-Phe-Pro-NH_2) have shown four different isomers generated from *cis-trans* isomerization of the Tyr-Pro and Phe-Pro amide bonds.[60] *N*-Methylated amino acids are commonly found in naturally occurring peptide antibiotics. Conformational calculations and NMR studies on Sar-Sar dipeptide **2.1** (where Sar is *N*-methylglycine) showed that the *cis* isomer is only 0.6 kcal/mol higher in energy than the *trans* (scheme 2.1).[61]

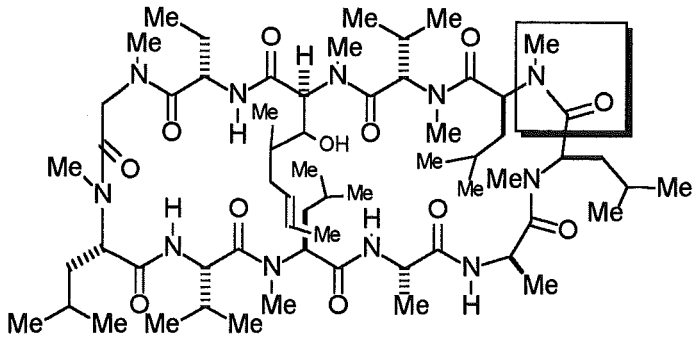


trans-**2.1** *cis*-**2.1**

Scheme 2.1: *Cis-trans* isomerization of **2.1**.

There are many examples of the *cis* amide configuration in biologically active peptides and analogues. In a series of angiotensin II **1.5** analogues, *trans* to *cis* isomerization of the His-Pro bond was observed by NMR,[62] when the compound was titrated in D_2O from acidic to neutral pD. The amount of *cis* isomer present was correlated with biological activity for the angiotensin II receptor in rat uterine. This suggested that the *cis* isomer was the one bound to the receptor and responsible for the observed activity. It has also been proposed[63] that the *cis-trans* isomerization of proline amide bonds might also play a role in transduction of membrane proteins. The isomerization of these membrane-bound proteins, and the resulting redirection of the protein chain, is proposed to provide the conformational change necessary for the reversible opening and closing of transport channels. Others suggested that *cis-trans* isomerization is also responsible for many of the slow kinetic events observed in enzyme kinetics and protein folding.[64]

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe **1.5**

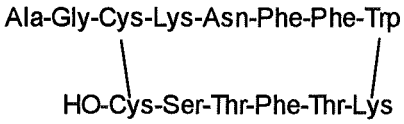


1.10

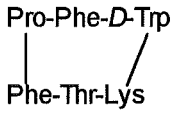
Cyclosporin A **1.10**, an immunosuppressant drug, is a conformationally constrained cyclic peptide containing seven *N*-methylated amide bonds. It is known that **1.10** inhibits a rotamase enzyme that catalyzes *cis-trans* isomerization of peptidyl-prolyl amide bonds. In non-polar solvents or in the solid state, it was observed that the hydrophobic peptide forms a twisted β-sheet structure with the MeLeu-MeLeu amide bond (shown in box) fixed in the *cis* conformation. In more polar solvents, like methanol or water, several conformations were observed with isomerization occurring about that amide bond.

Somatostatin **1.7** is a large cyclic peptide with an intramolecular disulfide bridge between two cysteine sidechains. Structure-activity relationship studies indicated that the Phe7-Trp8-Lys9-Thr10 sequence (the numbers indicate the position of the residue in the peptide sequence) was important for activity and that a β-turn within this sequence was required for receptor recognition. Based on these studies, conformationally restricted analogues with smaller ring structures to stabilize the turn, were synthesized,[65] such as **2.2**. Conformational analyses of these highly active analogues indicated that a *cis* amide bond between Phe-Pro was important for activity.

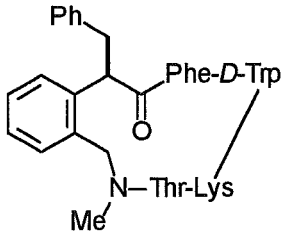
As a result of these and other studies, *cis* amide bond surrogates have been designed to force the *cis* configuration in peptide analogues, in order to mimic bioactive conformations and probe receptor specificity. Replacement of the amide bond with surrogates also improves the metabolic stability of potential drugs by preventing amide cleavage from degrading protease enzymes.



1.7

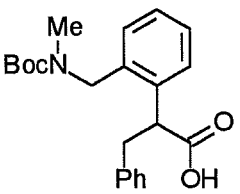


2.2

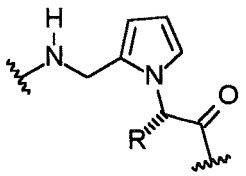


2.3

The dipeptide analogue **1.27**, containing an *ortho*-substituted benzene ring as a *cis* amide surrogate, was incorporated into the somatostatin analogue **2.2** as a replacement for the Phe-Pro sequence to give **2.3**. The analogue **2.3** showed substantial bioactivity compared to the parent somatostatin.[52] A *cis* amide surrogate based on **1.27** has also been incorporated into analogues of the RGD peptide, leading to an active inhibitor of cell adhesion,[66] and also into inactivators of trypsin-like proteases.[67]



1.27



1.28

The synthesis of a 1,2-disubstituted pyrrole-based dipeptide analogue **1.28** has been developed in our laboratories, as a generally applicable *cis* amide surrogate.[51] Analogues of the Gly-Gly, Gly-Ala, and Gly-Leu dipeptides were prepared and shown

to be easily extended in the *C* direction. By the correct choice of side chain groups, this surrogate can be applied specifically as a peptidomimetic.

trans *cis*

Scheme 2.2: Isomerization of an olefin.

The *trans* olefinic group[68] has been successfully used as a surrogate for a *trans* amide bond. However, the corresponding *cis* olefin is unsuitable as a *cis* amide surrogate due to the ease of *cis* to *trans* olefin isomerization (scheme 2.2). The CO-NH portion of the urethane bond of common *N*-terminal protecting groups such as Cbz and Boc has a high tendency to assume a *cis* configuration.[69a] A synthetic scheme was developed[69b] where a urethane bond was derived from the sidechain hydroxyl group of tyrosine (figure 2.1). The incorporation of these urethane linkages into backbones promoted cyclization of the compounds and gave rise to a family of macrocyclic peptide analogues, with numerous possible applications.

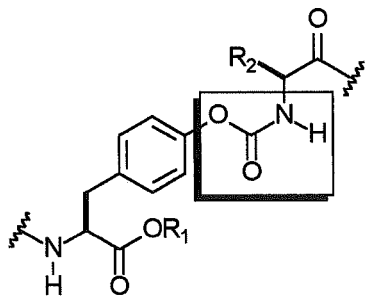
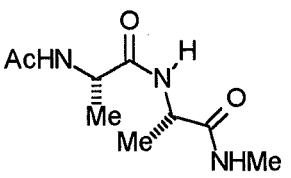


Figure 2.1: Tyrosine *cis*-urethane linkage (shown in box).

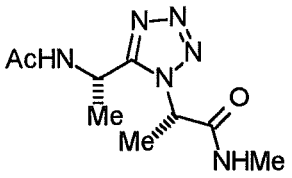
Smith, Hirschmann *et al.*[70] have developed very successful pyrrolinone-based peptidomimetics as mimics of β-strands. In these compounds 3,5,5-pyrrolin-4-one heterocycles are linked as replacements for the amide bonds of a native peptide to give

a novel completely non-peptidic scaffolding with attached sidechain groups (figure 2.2). Such compounds were shown to adopt the favorable pleated backbone conformations and antiperiplanar sidechain trajectories observed in some HIV protease inhibitors such as **1.19**. Pyrrolinone-based compounds also have improved pharmacological properties than the corresponding peptide-based peptidomimetics.

Marshall *et al.*[71] first proposed the tetrazole ring system as a *cis* amide bond surrogate to lock a dipeptide analogue in an equivalent configuration. The major concern in designing a surrogate is the amount of geometric and steric similarity to the *cis* amide bond. Using a novel procedure[50] for assessing conformational mimicry, it was shown that approximately 88% of the conformations accessible to the *cis* isomer of the dipeptide **2.4** are also accessible to the tetrazole analogue **2.5**. This index of conformational mimicry was measured by the ability of **2.5** to orient the backbone and sidechains, on either side of the tetrazole ring, in a similar manner to that of **2.4**. As the receptor-bound conformation of a target peptide is not always known, such statistical arguments for the suitability of analogues are useful. During these studies, the tetrazole **2.5** was found to have more conformational freedom than **2.4**. This probably reflects the increased valence angle αC–C=N of the tetrazole relative to the corresponding αC–C=O angle of the dipeptide. Based on these studies, Smith *et al.*[72] concluded that the tetrazole analogue is an excellent conformational mimic of the *cis* amide bond.



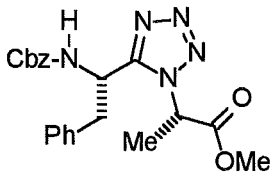
2.4



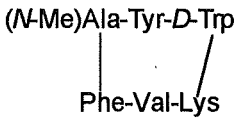
2.5

The tetrazole **1.26** was incorporated[73] into the cyclic somatostatin analogue **2.6** as a replacement for Phe-(*N*-Me)Ala. The resulting analogue showed nearly equivalent activity, indicating that it is the *cis* configuration of this amide bond that exists in the biologically active conformation of **2.6**. The tetrazole dipeptide **1.26** has also been incorporated into a substrate sequence of HIV protease as a replacement for the Tyr-

Pro scissile amide bond.[74] Molecular modelling of the tetrazole analogue and the substrate showed good correlation between the two conformations. However the tetrazole analogue was found not to inhibit the HIV protease. This result may indicate that the increased steric bulk of the tetrazole ring in the active site may prevent effective binding of the analogue.

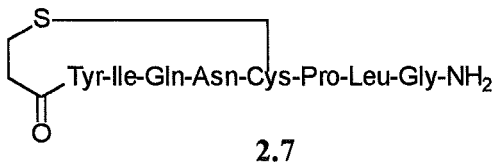


1.26

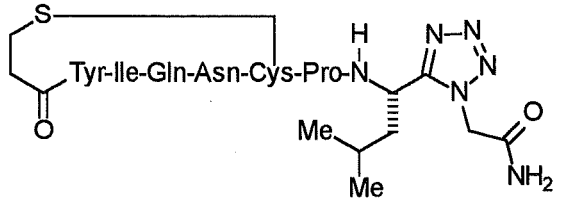


2.6

The tetrazole surrogate has also been incorporated as a replacement for the Leu-Gly amide bond of deaminooxytocin[75] **2.7** (see oxytocin **1.2** for comparison), to give **2.8** as a conformational probe of the importance of the *cis* configuration to this amide bond. Comparison of the bioactivity of **2.8** with that of a corresponding analogue incorporating a *trans* olefin surrogate suggested that a *trans* configuration was important to this amide bond. Replacement of the Pro-Pro and Ser-Pro amide bonds of bradykinin with tetrazole surrogates[76] gave analogues with greatly diminished activity relative to the parent peptides, suggesting either a *cis* configuration was not required or that the steric bulk of the tetrazole prevented key binding interactions.



2.7

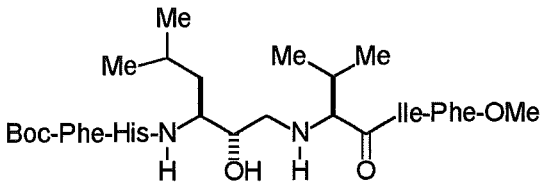


2.8

A classic strategy in the design of protease inhibitors is the incorporation of non-hydrolyzable dipeptide isosteres into the natural enzyme substrate at the P_1-P_1' cleavage site. Isosteric replacement of the scissile amide bond CO-NH (with CH_2NH in the case of the hydroxyethylamine isostere) increases the metabolic stability of the inhibitor.

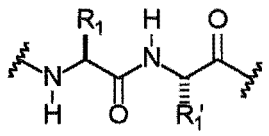
A number of dipeptide isosteres have been developed. The application of the transition state mimic principle has considerably increased the activity of these inhibitors. In such compounds, an sp^3 hydroxymethylene group is inserted in the centre of the isostere as a mimic of the tetrahedral transition state for hydrolysis of the P_1-P_1' amide bond. Enzymes bind more favourably to ligands resembling the transition state of the reaction than to those resembling substrate. The idea of using such isosteres was developed from the peptidomimetic natural product pepstatin **1.17**, which was discovered[37] as a potent mechanism-based inhibitor of aspartic proteases. Pepstatin contains the dipeptide isostere statine, from which other isosteres have been developed (figure 2.2).

The hydroxyethylamine isostere was first used to yield potent renin inhibitors,[77] such as **2.9**, by incorporation into the substrate sequence of angiotensinogen as a replacement for the scissile P_1-P_1' residues. However, due to its relatively large size and peptidic character, **2.9** possesses poor pharmacological properties and is not promising as an oral antihypertensive.

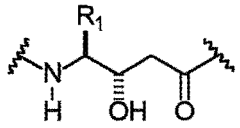


2.9

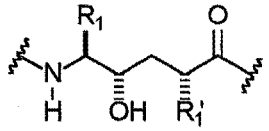
In comparison with other dipeptide isosteres (figure 2.2), such as statine and hydroxyethylene, the hydroxyethylamine isostere retains the favourable interactions of the hydroxymethylene function while causing less disturbance to the pattern of substrate-like binding. In particular, the amino function more closely mimics the position of the amide nitrogen of the substrate.



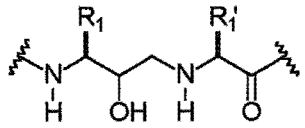
normal peptide substrate



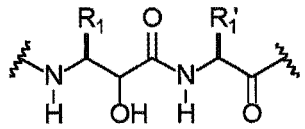
statine



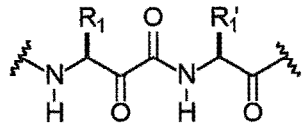
hydroxyethylene



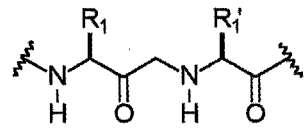
hydroxyethylamine



hydroxyamide



α-ketoamide

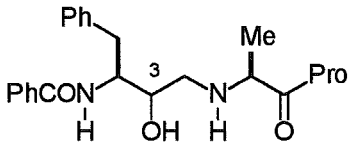


aminoketone

Figure 2.2: Non-hydrolyzable dipeptide isosteres.

The hydroxyethylamine isostere has also been used in inhibitors[78] of the metallo protease ACE, an important enzyme in the renin-angiotensin system. Incorporation into the known substrate *N*-benzoyl-Phe-Ala-Pro as a replacement for Phe-Ala gave potent inhibitor **2.10**. A series of compounds based on **2.10** were synthesized to investigate the consequences of structural and configurational modifications to activity. The configuration of the hydroxymethylene group was found to be important with the

(*3R*)-epimer of **2.10** possessing 400-fold greater activity than the (*3S*)-epimer. The removal of the hydroxyl group altogether resulted in extremely poor activity. The secondary amine of the hydroxyethylamine core was also shown to be important for activity when replaced by an ether function, resulting in a poorly active analogue. This suggested that the amino group acts as an important hydrogen bond acceptor-donor in the active site.



2.10

The hydroxyamide[79] and the α-ketoamide[80] dipeptide isosteres (figure 2.2) have been used successfully in potent HIV protease inhibitors (for a discussion see the following section on HIV protease inhibition). The extra amide bonds of these structures may make them less hydrolytically stable than the hydroxyethylamine structure, however they provide more possible hydrogen bonding interactions with a receptor. It was proposed that α-ketoamide based inhibitors of HIV protease are hydrated within the active site to form a stabilized hydrate which is a good transition state mimic (figure 2.3).

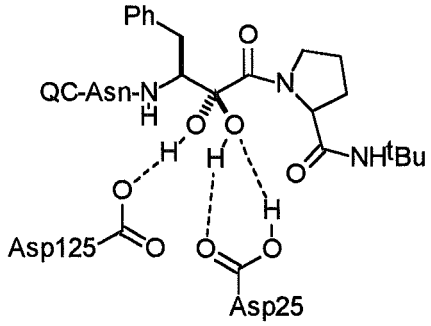
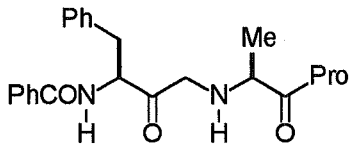
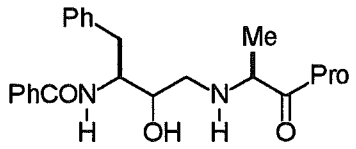


Figure 2.3: Proposed hydrated α-ketoamide in the active site.

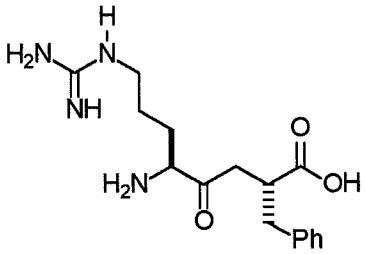
Related to the hydroxyethylamine isostere is the aminoketone structure (figure 2.2), resulting from conversion of the hydroxyl group of the hydroxyethylamine isostere to a ketone. The resulting ACE inhibitor **2.11** was synthesized[78] to compare with **2.10** and was found to be more potent. The mode of action of **2.11** would be similar to an α-ketoamide (figure 2.3), where nucleophilic attack on the ketone in the active site, would lead to a hemi-ketal transition state mimic. The ketomethylene ($COCH_2$) component of the aminoketone isostere is found in the naturally occurring aminopeptidase inhibitor arphamenine, and has also been incorporated into inhibitors of a number of other enzymes, including aminopeptidase[81] and renin.[82]



2.11

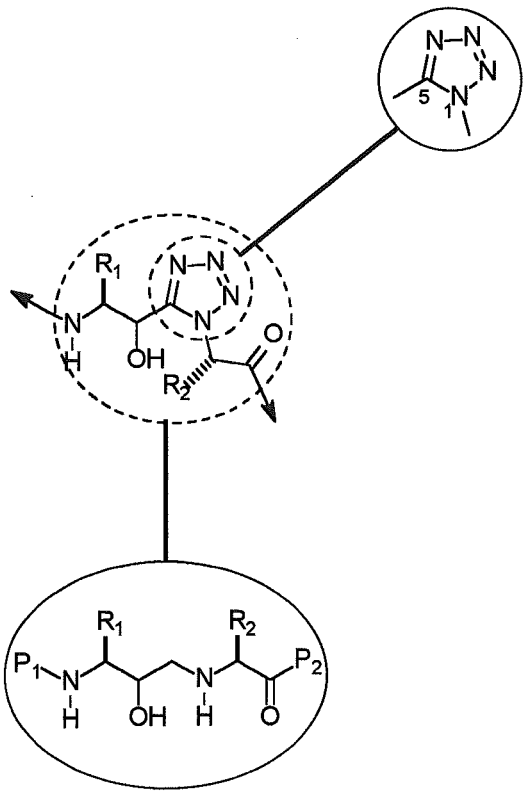


2.10



arphamenine

cis-Hydroxyethylamine Isostere



cis amide bond surrogate

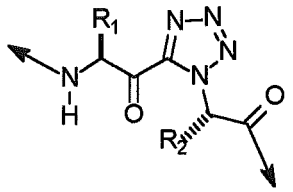
cis-hydroxyethylamine isostere **2.12**

hydroxyethylamine dipeptide isostere

Figure 2.4: Design of our *cis*-hydroxyethylamine isostere.

The subject of a significant part of the research undertaken in this thesis is a peptidomimetic structure combining the design principles of a *cis* amide bond surrogate and the hydrolytically stable hydroxyethylamine dipeptide isostere. This new *cis*-hydroxyethylamine isostere **2.12** incorporates a tetrazole ring in the centre of the dipeptide analogue to conformationally constrain the 1 and 5 substituents in a *cis* configuration (figure 2.4). The isostere **2.12** is generally applicable in the design of peptidomimetics by selection of suitable natural or unnatural sidechains at R_1 and R_2, and peptidic or non-peptidic extensions in the *N* and *C* directions.

The isostere **2.12** can also be modified to give an α-ketotetrazole **2.13**, where the hydroxyl group is converted to a ketone. This modification combines the design principles of the α-ketoamide and amino ketone isosteres discussed above.



2.13

One envisaged application for isosteres **2.12** and **2.13** is in the design of HIV protease inhibitors, based on the known binding conformation of JG365 **1.19** (see following for a discussion).

HIV Protease Inhibition

The Human Immunodeficiency Virus (HIV) infects almost 20 million people worldwide and leads to the deadly disease AIDS. Enormous research is being directed toward developing therapeutic drugs to treat HIV. Until recently AIDS has been an inevitably fatal, incurable disease. However this year, exciting new clinical results have emerged. Powerful combinations of HIV protease and HIV reverse transcriptase inhibitors have been shown to reduce viral levels in the blood of infected patients to undetectable levels. These combinations of drugs include the more established HIV reverse transcriptase inhibitors, such as AZT and 3TC, and three recently approved HIV protease inhibitors, including **1.20**.

A second development is the discovery of chemokines,[83] compounds secreted by certain white blood cells that potently inhibit HIV replication. It has been shown that a chemokine receptor has a critical role in HIV's ability to infect cells. Complete eradication of the virus from patients is now a possibility.

The HIV protease[84] is a virally encoded enzyme that cleaves viral polypeptides transcribed from the *gag* and *pol* genes, late in the viral replicative cycle. Although it can cleave a number of specific peptide bonds, the HIV protease is unusual in being able to cleave the Phe-Pro and Tyr-Pro sequences of its substrate. The amide bonds of proline residues are not very susceptible to cleavage by other mammalian endopeptidases. The protease is essential for the assembly and maturation of infectious virions. Inactivation of HIV protease, by a single mutation of the Asp25 residue in the

active site, resulted in non infectious virions and lead to the targeting of HIV protease inhibition as a viable strategy for treatment of the virus. The HIV protease belongs to the class of aspartic proteases (see chapter one) that includes renin and pepsin and which catalyzes the hydrolysis of amide bonds via aspartate residues in the active site. An enormous effort has been directed toward developing renin inhibitors as antihypertensive drugs, based on the mechanism of action of aspartic proteases. Many of the principles established in these efforts have now been applied to the design of HIV protease inhibitors.

The rational design of inhibitors based on isosteres such as those shown in figure 2.2, has been greatly facilitated by the availability of three-dimensional X-ray crystal structures of HIV protease in its native form[85] and in a number of inhibitor complexes.[86] The HIV protease (figure 2.5) is a homodimer comprising two identically folded ninety-nine amino acid subunits that form a hydrophobic active site cavity. This C_2 symmetry is a unique feature of retroviral proteases. Two conformationally flexible flaps are able to close around the substrate upon binding. This is shown in figure 2.5, by a comparison of the conformation of free enzyme with that of enzyme bound to an inhibitor.[87] The two catalytic aspartate residues are centered in a cylindrical substrate binding groove. One residue acts as a base, initiating nucleophilic attack on the scissile amide bond of the substrate by an active site-bound water molecule. The other aspartate residue acts as an acid, stabilizing the carbonyl oxygen in the tetrahedral intermediate (see scheme 1.1). In order for rational drug design to succeed, it has been important to characterize the structures of the enzyme and its inhibitor complexes. A superimposition[87] of the X-ray crystal structures of twelve different HIV protease-bound inhibitors is shown in figure 2.6. Figure 2.6 demonstrates how a number of different molecules can adopt the same conformation and bind to the enzyme. It also defines the S_3-S_3' pockets of the enzyme and the shape and binding properties of the active site.

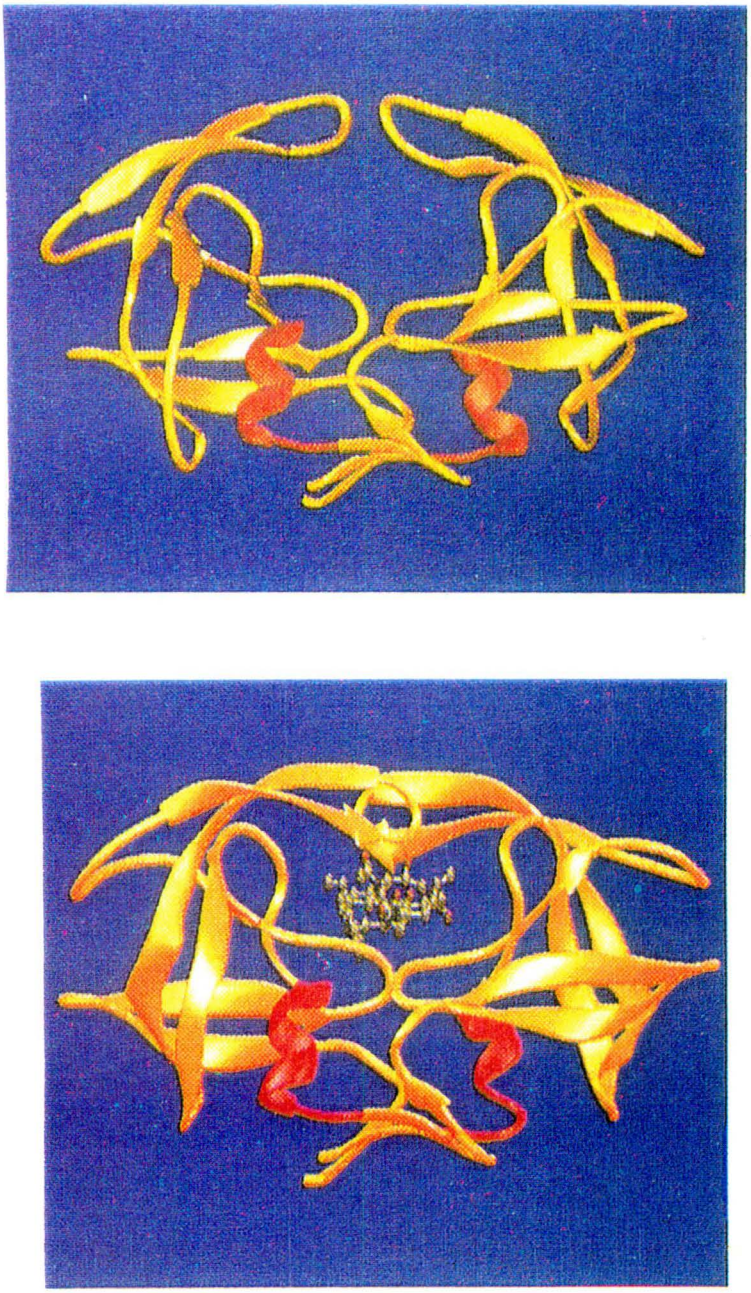
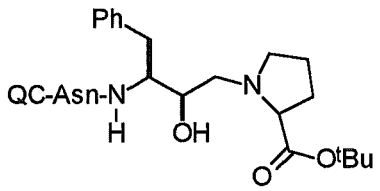
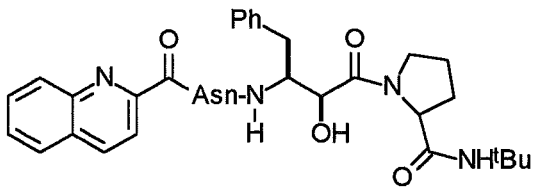


Figure 2.5: Computer generated images[87] of the structure of native HIV protease (top) versus inhibitor (MVT 101)-bound HIV protease (bottom). Note that the flaps are opened in the native which close upon the inhibitor.

Figure 2.6: Superimposition of the X-ray crystal structures of 12 enzyme-bound inhibitors.[87]

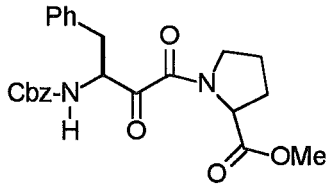


2.14



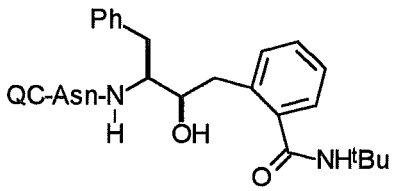
2.15

The hydroxyamide (norstatine) isostere (figure 2.2) was incorporated into potent HIV protease inhibitors,[79] such as **2.15**, to compare with related hydroxyethylamine-based compounds, such as **2.14**. The two types of inhibitors differ by one function, a carbonyl group in **2.15** which is a methylene group in **2.14**. The extra amide bond of **2.15** may make it less hydrolytically stable than **2.14**, however it provides more possible hydrogen bonding interactions in the active site. Compounds based on **2.15** were found to be 10-20 times more potent inhibitors of HIV protease than compounds based on **2.14**.



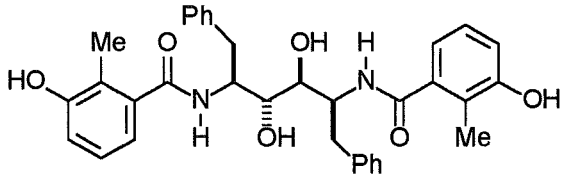
2.16

The α-ketoamide dipeptide isostere[80] (figure 2.2) appears to be more potent than any other known mechanism-based isostere in HIV protease inhibitors. In general, activated ketones have been used in inhibitors of aspartic and serine proteases. HIV protease inhibitors such as **2.16** were proposed to form stabilized hydrates within the active site (see figure 2.3).



2.17

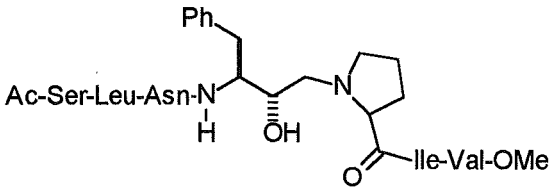
Other isosteric P_1-P_1' replacements have been employed and exhibit potent activity. Benzamide-based inhibitor **2.17** was developed by Kaldor *et al.*[88] at the Lilly laboratories based on enzyme-inhibitor X-ray crystal structures. Molecular modelling calculations indicated that the benzamide carbonyl would be able to rotate out of the resonance plane of the adjacent benzene ring to enable a crucial hydrogen bonding interaction with a highly localized water molecule in the flap region. This prediction was later confirmed by the X-ray crystal structure of the HIV protease-**2.17** complex. A number of benzene-substituted derivatives with varying activities were also prepared, to investigate the dimensions of the benzamide binding pocket at S_1'.



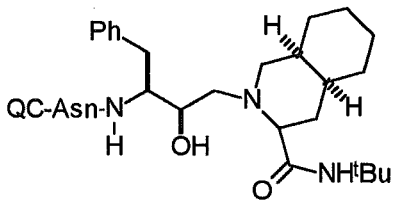
2.18

In a novel approach, HIV protease inhibitors were designed which mimic the approximate C_2 symmetry of the enzyme. Kempf *et al.*[42] imagined placing the C_2 symmetry axis of the enzyme through the proposed tetrahedral intermediate for amide hydrolysis and performing the C_2 operation on either the P_1 or P_1' regions of the substrate (see figure 1.7). Such structures may provides advantages over traditional substrate-based inhibitors, in terms of potency and selectivity for HIV protease over other aspartic proteases. An X-ray crystal structure of the HIV protease-**2.18** complex showed an extended binding conformation, spanning the S_2 to S_2' subsites.[89] Several C_2 symmetric inhibitors are undergoing clinical trials.

A key feature of all the potent peptide-based inhibitor-HIV protease complexes is the hydrogen bonding from the two central carbonyls of the inhibitor to a water molecule which bridges to the Ile50 and Ile150 residues in the flaps of the enzyme.



1.19



1.20

Potent and selective HIV protease inhibition is possible by mimicking the Phe-Pro cleavage site unique to retroviral proteases. Since this cleavage site is rare in mammalian endopeptidases, such inhibitors have a selective advantage for HIV protease and are not as easily degraded by other enzymes. Incorporation of the hydroxyethylamine dipeptide isostere into the minimum substrate peptide sequence required for recognition gave **1.19**.[41,90] It is a potent inhibitor *in vitro* but failed to inhibit HIV in cell culture, possibly due to its largely peptidic character. Therefore, strategies were developed to increase favourable pharmacological properties. Smaller inhibitors, occupying two or three chain positions in the active site and having reduced or no peptidic character were targeted. Potent hydroxyethylamine-based inhibitor **1.20** was developed[41] by the Roche laboratories as a result of substantial structure-activity correlations, and exhibits strong antiviral activity. It was recently approved for marketing in the USA under the name Saquinavir, and is one on the drugs involved in exciting new clinical results.

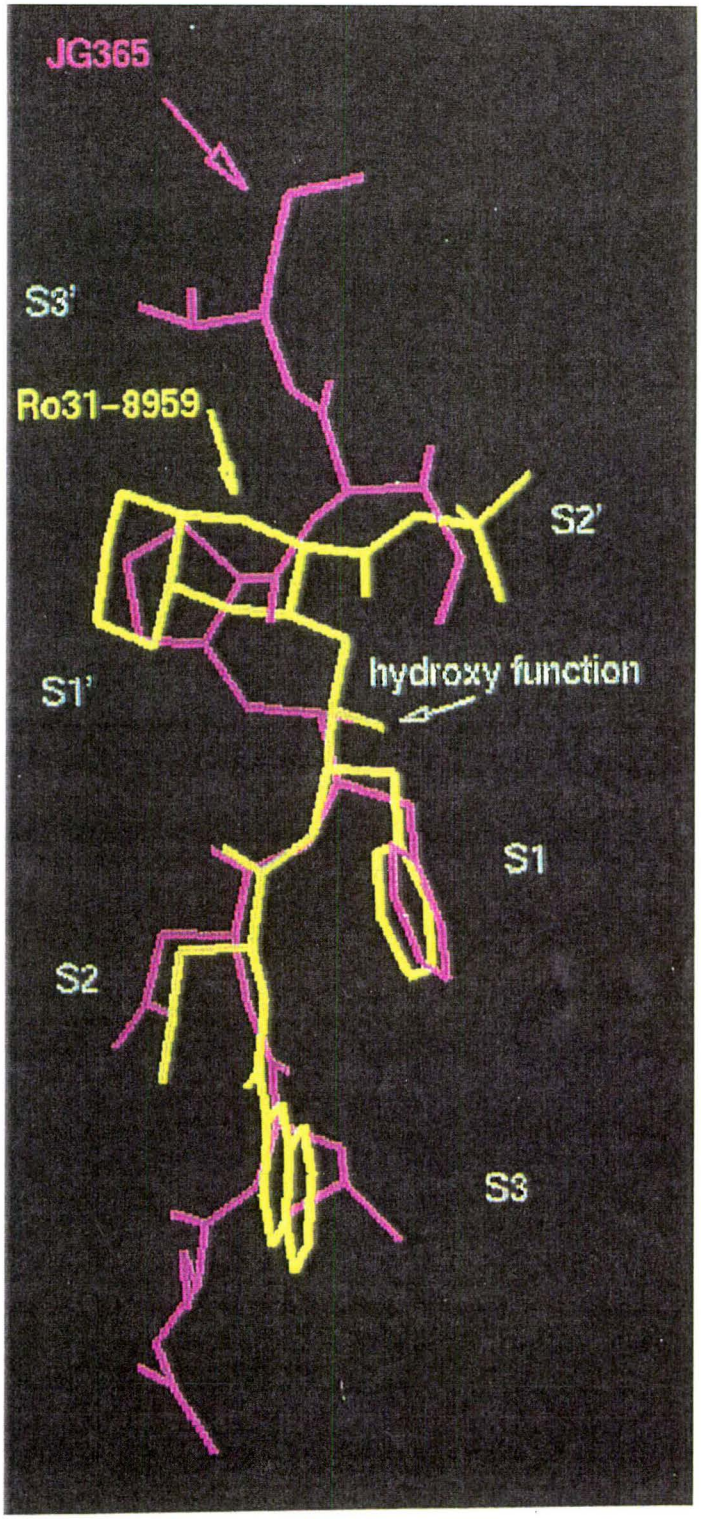
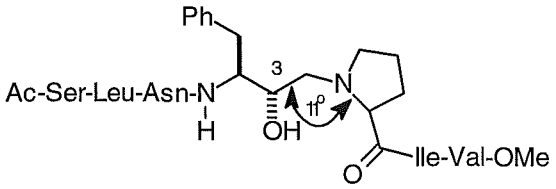


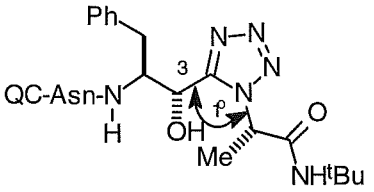
Figure 2.7: Superimposition of enzyme-bound **1.19** (JG365, X-ray) and **1.20** (Ro31-8959, modelled).[91]

Two distinct inhibitor binding modes[91] are observed in the X-ray crystal structures of the HIV protease-inhibitor complexes of **1.19**[92] and **1.20** (figure 2.7). In **1.19** the Ile-Val (P_2'-P_3') residues occupy the S_2'-S_3' enzyme subsites as expected and force a favourable (*S*)-OH configuration on the transition state mimic. However, in **1.20** the *C*-terminal *tert*-butyl amide group fits well into the S_2' subsite with the large decahydroisoquinoline (Diq) ring structure occupying the entire S_1' subsite, with some S_3' interactions. This binding mode shows a clear preference for the opposite (*R*)-OH configuration at the transition state mimic. Extension of **1.20** to P_3' showed a marked decrease in activity. Similarly, inhibitors based on **1.19**, lacking a P_3' ligand and with Pro at P_1', show moderate potency and a slight preference for (*R*)-OH, indicating the second binding mode. The superimposition of the HIV protease-bound structures of **1.19** and **1.20** in figure 2.7, clearly shows the two distinct binding modes with differing backbone conformations between the hydroxyl groups and the prolyl or Diq rings. The backbone of **1.19** adopts a *cis* geometry about this region while **1.20** has a *trans* arrangement at the equivalent position from the hydroxyl group to the Diq ring.

It was envisaged that our designed isostere **2.12** would be able to bind by the first mode, adopting a bend *cis*-type conformation similar to that of **1.19**. Molecular modelling studies of compound **2.19** (figure 2.8), based on our designed isostere **2.12**, with a (*R*)-configuration at the C3 center, were done using the Macromodel software. A Monte Carlo conformational search of an energy minimized structure in an Amber forcefield, followed by further low gradient minimizations and local minima/maxima testing, gave a global energy minima. This structure was flexibly superimposed onto the enzyme-bound X-ray crystal structure of **1.19**, to generate figure 2.9 (front view) and figure 2.10 (side view). An identical modelling study was done on the epimer **2.20**, with a (*S*)-configuration at C3. The superimposition of modelled **2.20** on **1.19** is shown in figure 2.11. It should be noted that although the assignments of (*3S*)-**1.19** and (*3R*)-**2.19** are different, they do have they same relative C3 configurations.



1.19



2.19

Figure 2.8: Relevant torsion angles of **1.19** and modelled **2.19**, from the superimposition in figures 2.9 and 2.10.

Figures 2.9 and 2.10 suggest that the modelled conformation of **2.19** compares well with the bioactive conformation of inhibitor **1.19**. The torsion angle around the tetrazole ring (1°) is restricted to a value close to the corresponding near planar torsion angle (11°) adopted by the backbone of enzyme-bound **1.19** (figure 2.8). The side view in figure 2.10 shows clearly the *cis* conformation of the two superimposed backbones of **2.19** and **1.19**. This particular torsion angle of **1.19** would not be constrained in solution and only adopt the planar *cis* arrangement upon binding to HIV protease. It was envisaged that the conformational restriction imposed by the tetrazole ring, would be the basis for mimicking the bioactive conformation of **1.19**, and result in comparable HIV protease inhibition.

Figure 2.9: Front view of the superimposition of modelled **2.19** (in red) on the enzyme-bound X-ray crystal structure of **1.19** (in blue) (only the P_1-P_1' regions are shown, for clarity).

Figure 2.10 also shows that the two central carbonyl groups (pointing out to the left) are in a suitable position to form crucial hydrogen bonds with an active site-bound water molecule (not shown). The P_1 phenylalanine side chain groups can also be seen projecting into where the S_1 pocket of the enzyme would be. It is evident from the modelling studies that the P_3-P_1 ligands of **2.19** are able to occupy the same enzyme subsites as **1.19**. However, the steric bulk of the tetrazole ring may be a hindrance to binding if it is unable to project into the S_1' subsite occupied by the prolyl and Diq ring structures of **1.19** and **1.20** (figure 2.7). Another important factor in enzyme binding is the choice of a P_1' ligand which will completely fill the S_1' binding pocket. The large Diq ring of **1.20** completely fills the S_1' subsite, as observed in the X-ray crystal structure of the HIV protease-**1.20** complex, and imparts greater activity

compared with the smaller prolyl ring of **1.19**. On this basis, it may be favourable in the future to alter the P_1' methyl side chain of our designed**2.19** to a larger group.

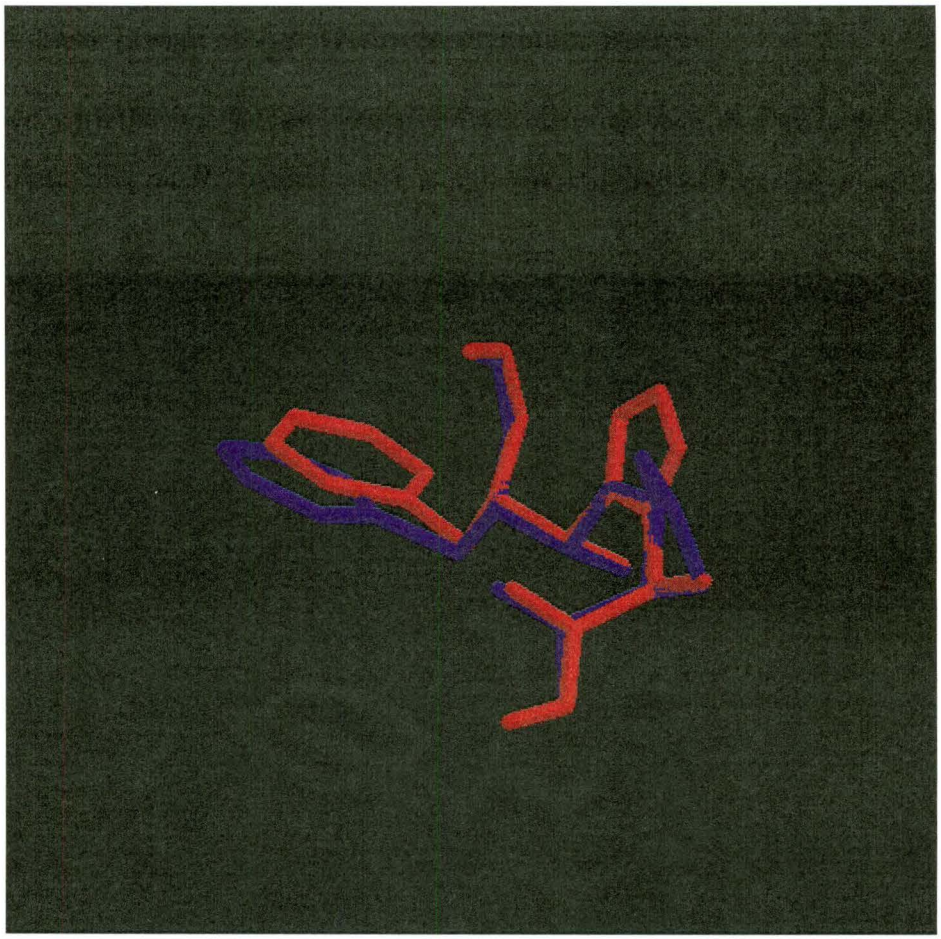


Figure 2.10: Side view of the superimposition of modelled **2.19** (in red) on the enzyme-bound X-ray crystal structure of **1.19** (in blue).

The (*3S*)-epimer of **2.19**, compound **2.20** is shown in figure 2.11 superimposed on structure **1.19**. Figure 2.11 clearly shows the C3-hydroxyl group of modelled **2.20** projecting in the opposite direction to the corresponding hydroxyl group of **1.19**. Comparison to figure 2.9 where the hydroxyl groups of modelled **2.19** and **1.19** are superimposed, clearly suggests a preference for the (*R*)-configuration at C3, in order for our designed inhibitors, based on **2.12**, to assume the known bioactive conformation of **1.19**.

Figure 2.11: Superimposition of modelled **2.20** (in red), with a (*S*)-configuration at C3, on the enzyme-bound X-ray crystal structure of **1.19** (in blue) (only the P_1-P_1' regions are shown, for clarity).

Traditionally, the activity of dipeptide isosteres has been enhanced by the addition of amino acid residues to both the *N* and *C* terminals, to improve recognition in the active site. Although this approach has been used to develop potent enzyme inhibitors, they generally exhibit poor pharmacological properties due to their peptidic character. Extensive research[93] is being done to develop non-peptidic ligands for the traditional P_3-P_2' positions of potent HIV protease inhibitors, which will show potent activity in clinical trials.

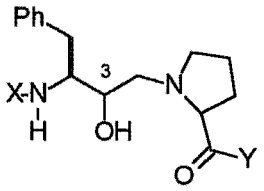
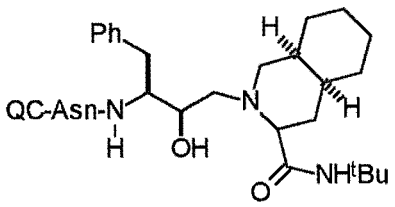
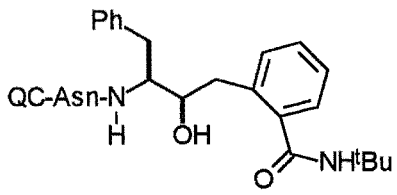


Table 2.1: HIV protease activity of **1.19** based inhibitors.

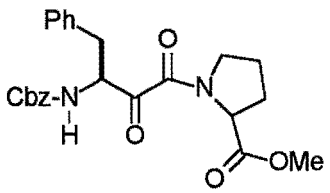
	X	Y	C3	IC_{50} (nM)	Ref
1.19	Ac-Ser-Leu-Asn	Ile-Val-OMe	*S*	0.24	[41]
2.21	Ac-Ser-Leu-Asn	Ile-Val-OMe	*R*	20	[41]
2.22	Cbz-Asn	O^{t}Bu	*S*	300	[41]
2.23	Cbz-Asn	O^{t}Bu	*R*	140	[41]
2.14	QC-Asn	O^{t}Bu	*R*	23	[41]
2.24	Cbz-Asn	NH^{t}Bu	*R*	210	[41]
2.25	Cbz	O^{t}Bu	*R*	6500	[41]
2.26	Ac-Leu-Asn	Ile-Val-OMe	*RS*	K_i 21 nM	[79]
2.27	Ac-Leu-Asn	Ile-OMe	*RS*	K_i 4520 nM	[79]



1.20 IC_{50} 0.23 nM

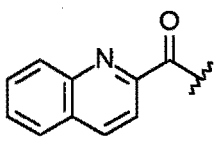


2.17 IC_{50} 1.5 nM



2.16 IC_{50} 405 nM

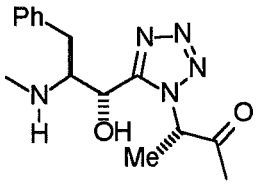
QC =



In order to make comparisons to the activity of known potent inhibitors of HIV protease (table 2.1), suitable P_3-P_3' ligands were chosen for our designed isostere **2.12** (table 2.2). The phenylalanine side chain at P_1 and asparagine residue at P_2 are favoured in all the inhibitors. The 2-quinolinylcarbonyl (QC) ligand at P_3 seems favoured over Cbz by comparison of the activities of **2.14** and **2.23** (table 2.1). Inhibitors **1.20** and **2.17** also favour QC-Asn at P_3-P_2. Ideally, to promote the binding mode of **1.19** (which our modelled compound **2.19** mimics), ligands extending from P_2' to P_4', such as Ile-Val-OMe, would be more favourable (as discussed above). A comparison of the activities of **2.26** and **2.27** (table 2.1), shows how extension of the inhibitor by one residue in the *C*-direction, can significantly increase the activity. However initially, a simpler, more synthetically accessible inhibitor was targeted with O^{t}Bu or NH^{t}Bu at P_2' (table 2.2), suitable for comparison to **2.22**.

An inhibitor with Cbz at P_2 and OMe at P_2' (table 2.2) was also targeted in order to compare the intrinsic potency of our designed *cis*-hydroxyethylamine isostere **2.12**, with the most potent known α-ketoamide core structure of **2.13**.

Table 2.2: Possible P_3-P_3' ligands for isostere **2.12**.

	P_3	P_2	P_1-P_1'	P_2'	P_3'-P_4'
2.19	QC	Asn		NH^{t}Bu	
	Cbz	Asn		O^{t}Bu	
	Cbz	Asn		Ile	Val-OMe
		Cbz		OMe	

These proposed comparisons enable a further probe of the effectiveness of our designed structure **2.12** to the application of HIV protease inhibition. It was envisaged that, from these initial **2.12**-based compounds, further structural modifications such as suitable extension from P_2' to P_4' and reduction of the compound's peptidic character, could be made to maximize the activity.

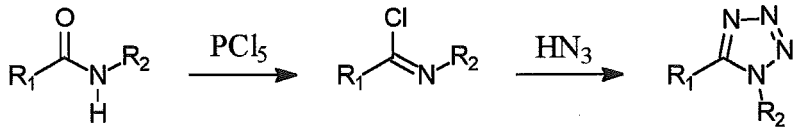
Conclusion

The new, generally applicable peptidomimetic *cis*-hydroxyethylamine structures **2.12** and **2.13** were designed based on the design principles of a *cis* amide surrogate and a hydrolytically stable dipeptide isostere. A possible application of this system in the inhibition of HIV protease was discussed, based on the known binding conformation of an existing potent inhibitor. A series of compounds based on **2.12** were targeted for synthesis with a view to comparing their activities with those of known potent inhibitors.

Chapter Three

Synthesis of the *cis*-Hydroxyethylamine Isostere

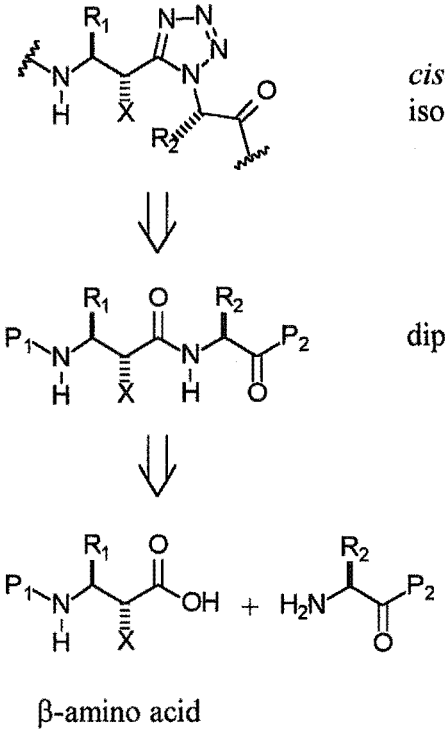
1,5-disubstituted tetrazoles were first proposed by Marshall *et al.*[71] as a *cis* amide bond surrogate (see chapters one and two). A tetrazole is conveniently prepared from the corresponding amide by reaction with phosphorus pentachloride (PCl_5) to give the imidoyl chloride, followed by reaction with hydrazoic acid (HN_3) (scheme 3.1). Zabrocki *et al.*[76] have developed this procedure to prepare 1,5-disubstituted tetrazole dipeptide analogues suitable for incorporation into larger peptides.



amide imidoyl chloride tetrazole

Scheme 3.1: Formation of a tetrazole.

Based on these procedures, a retrosynthesis of the target *cis*-hydroxyethylamine isostere (see chapter two for a discussion of the design) was devised (scheme 3.2). The tetrazole **2.12** would be derived from the corresponding protected unnatural dipeptide **3.1** and then extended in the *N* and *C* directions as required. The unnatural dipeptide precursor would be prepared by a coupling of the corresponding *N*-protected β-amino acid with a suitable carbonyl-protected α-amino acid.



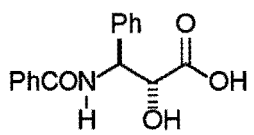
cis-hydroxyethylamine isostere **2.12**

dipeptide **3.1**

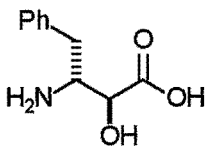
β-amino acid

Scheme 3.2: Retrosynthetic scheme for the target **2.15** (X is OH or H).

Unusual β-amino acids and their α-hydroxy derivatives occur in a diverse range of natural products with significant biological activities. The potent anticancer drug taxol **1.8** (see discussion in chapter 1) contains the α-hydroxy-β-amino acid **3.2**, which is essential to its biological activity.[94] Bestatin is well known as an immune-response modifier and inhibitor of aminopeptidase B.[95] The *N*-terminal residue of bestatin is composed of the α-hydroxy-β-amino acid **3.3**. The configurations of both **3.2** and **3.3** are crucial to the biological activities of their parent molecules.



3.2



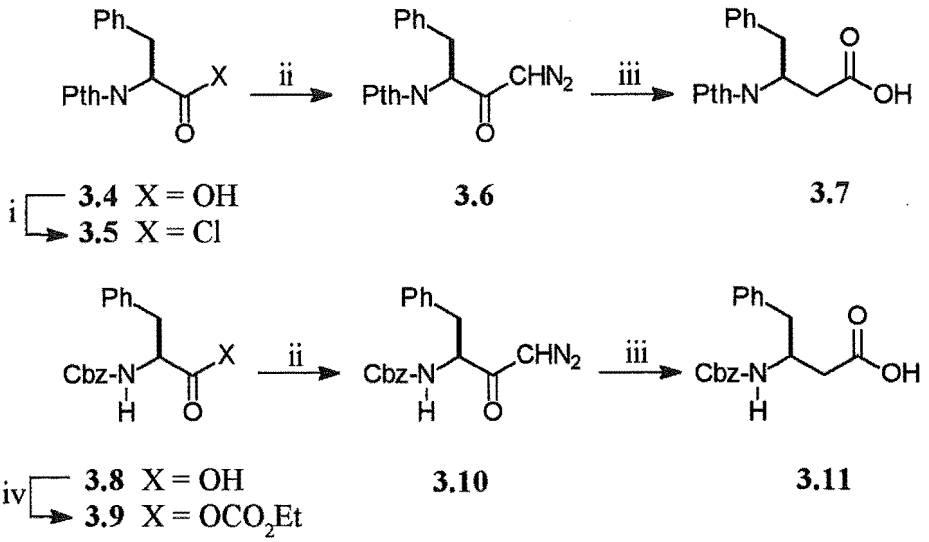
3.3

Apart from their intrinsic properties, β-amino acids are also useful synthetic intermediates in preparing β-lactams,[96] piperidines,[97] indolizidines,[98] and modified

peptides.[99] As a result of their undeniable chemical and pharmaceutical potential, enormous efforts have gone into the development of enantioselective syntheses of β-amino acids[100] and their α-hydroxy derivatives.[101]

Synthesis of a β-Amino Acid

Arndt-Eistert homologation of a *N*-protected α-amino acid gives the corresponding β-amino acid via the Wolff rearrangement of an α-diazoketone intermediate. In the 1940s, it was shown that the Wolff rearrangement of α-diazoketones, containing chiral centers adjacent to the carbonyl, occurred with retention of configuration. This opened up an ideal synthetic route to homologated α-amino acids from cheap and enantiomerically pure starting materials.

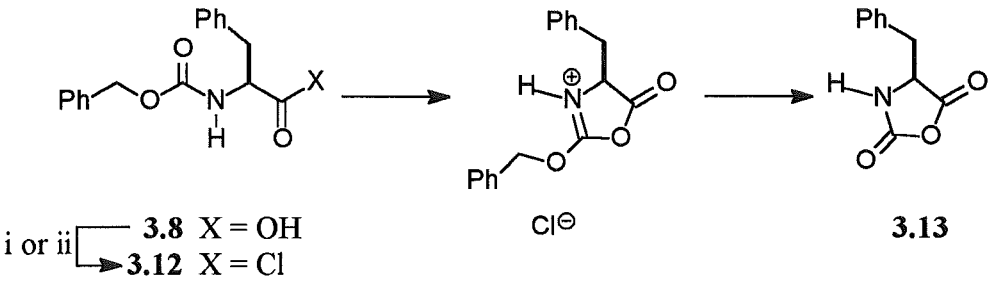


Scheme 3.3: **i**, oxalyl chloride, DMF, benzene, rt, 1 h; **ii**, CH_2N_2, ether, 5 °C, 18 h; **iii**, Ag_2O, Na_2CO_3, $Na_2S_2O_3$, dioxane, water, reflux or 78-85 °C, 1.5-2 h, then 10% HNO_3; **iv**, EtOCOCl, Et_3N, ether, THF, -20 °C, 15 min.

N-Phthalyl and *N*-benzyloxycarbonyl-*L*-β-homophenylalanine (Pth-*L*-βPhe **3.7** and Cbz-*L*-βPhe **3.11**) were prepared via Wolff rearrangement of the corresponding α-diazoketones (step iii, scheme 3.3). A benzene solution of Pth-*L*-Phe **3.4** was treated

with oxalyl chloride and *N*,*N*-dimethylformamide (DMF) to generate the acid chloride **3.5**, which was reacted *in situ* with diazomethane to give crystalline α-diazoketone **3.6** in 93% yield.

Chaturvedi *et al.*[102] described the preparation of *N*-Cbz α-diazoketone **3.10** (scheme 3.3), via the acid chloride. However, attempts to prepare acid chloride **3.12** by reaction of **3.8** with oxalyl chloride or PCl_5 resulted in large yields of phenylalanine-*N*-carboxyanhydride **3.13** (scheme 3.4). This intramolecular cyclization is well known.[103] The treatment of *N*-Cbz-amino acids with thionyl chloride, if carried out above room temperature is a convenient method for the preparation of *N*-carboxyanhydrides (Leuch's anhydrides).

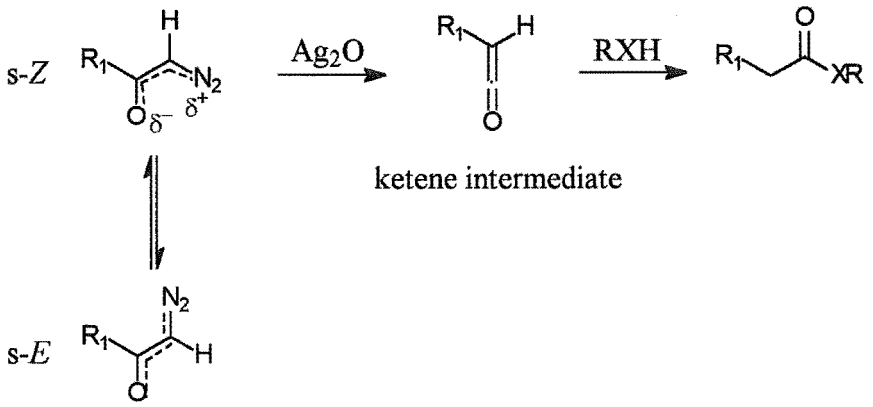


Scheme 3.4: **i**, oxalyl chloride, DMF, benzene, rt, 1 h; **ii**, PCl_5, ether, rt, 15 min.

A superior procedure[104] for the preparation of *N*-Boc and *N*-Cbz-amino acid α-diazoketones is via the corresponding mixed anhydrides. The treatment of Cbz-*L*-Phe **3.8** with ethyl chloroformate and triethylamine followed by *in situ* reaction of the mixed anhydride intermediate with diazomethane gave the α-diazoketone **3.10** in 93% yield (scheme 3.3, steps iv and ii).

The α-diazoketones **3.6** and **3.10** were dissolved in dioxane-water and heated in the presence of silver(I) oxide to give the corresponding *N*-protected β-amino acids **3.7** and **3.11**, in 65% and 56% yields, respectively (scheme 3.3, step iii). A Wolff rearrangement of a variety of readily accessible α-diazo carbonyl compounds opens up many preparative applications which after 90 years has generated enormous theoretical and mechanistic interest. The rearrangement of α-diazoketones proceeds via ketene intermediates, with elimination of nitrogen and R_1 group migration. Thermal initiation

of the rearrangement is catalyzed by metal ions such as Ag(I). A concerted mechanism has been proposed[105] for the ketene formation (scheme 3.5), from the s-(*Z*) form of the α-diazoketone, where the leaving group N_2 and migrating group R_1 are in an ideal antiperiplanar geometry. A competing, non-concerted, carbenic pathway to the ketene has also been proposed. The ketene intermediate can then be trapped by a variety of weak acids RXH (where RX can be HO, RO, RNH, HONH, R_2N or RS) to give a homologated acid or derivative. The ketene intermediate can now also be trapped by the amine functionality of an amino acid derivative[100] to give a dipeptide-based product. This eliminates the extra step of coupling to a β-amino acid.



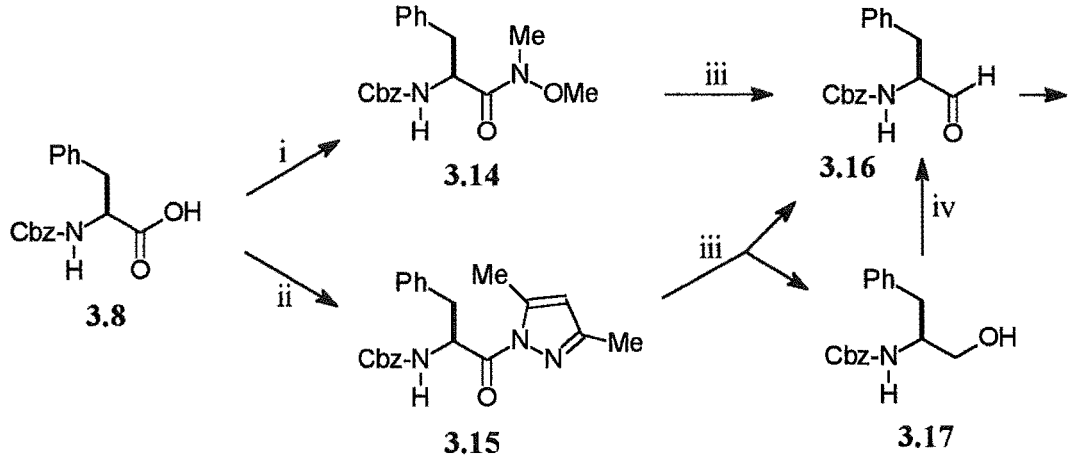
Scheme 3.5: Wolff rearrangement of an α-diazoketone.

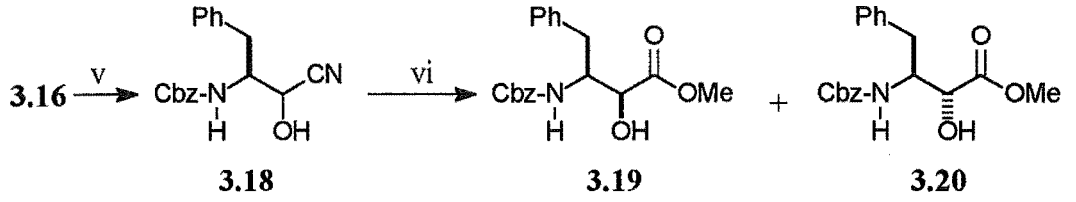
Synthesis of an α-Hydroxy-β-amino Acid

The methyl esters **3.19** and **3.20** were synthesized from **3.8** by a modified procedure (scheme 3.6) to that developed by Herranz *et al.*.[106] Using this procedure, the aldehyde **3.16** , prepared in two steps from Cbz-*L*-Phe **3.8**, was converted to a 1:1 mixture of the cyanohydrin diastereoisomers **3.18**. Hydrolysis then gave the methyl esters **3.19** and **3.20**.

The key aldehyde **3.16** was prepared from Cbz-*L*-Phe **3.8**, via a reduction with lithium aluminium hydride of either **3.14** or **3.15**. A benzotriazolyltris(dimethylamino)phosphonium hexafluorophosphate (BOP)-mediated coupling of **3.8** with *O*,*N*-dimethylhydroxylamine[107] gave **3.14** in 85% yield, while a

similar dicyclohexylcarbodiimide (DCC)-mediated coupling of **3.8** with 3,5-dimethylpyrazole gave **3.15** in 86% yield. Reduction of **3.14** with lithium aluminium hydride, at -50 °C, for fifteen minutes gave the aldehyde **3.16** in 77% yield. However, a similar reduction of **3.15** gave a mixture of **3.16** and the alcohol **3.17** in 49% and 29% yields, respectively. The alcohol **3.17** was formed by further reduction of the aldehyde **3.16** with excess lithium aluminium hydride. The alcohol **3.17** was reoxidized to **3.16**, in 98% yield, using Dess-Martin periodinane.[108]

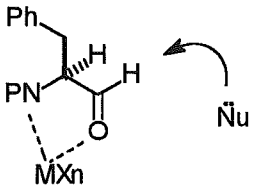




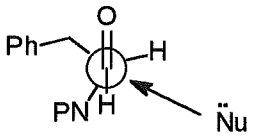
Scheme 3.6: **i**, BOP, Et_3N, *O,N*-dimethylhydroxylamine, CH_2Cl_2, rt, 18 h; **ii**, 3,5-dimethylpyrazole, DCC, $CHCl_3$, -10 °C, 1 h, rt, 2 d; **iii**, $LiAlH_4$, THF, -50 °C, 15 min, -50 to 0 °C, 20 min; **iv**, Dess-Martin periodinane, CH_2Cl_2, rt, 1 h; **v**, KCN, EtOAc, water, rt, 18 h; **vi**, MeOH, ether, $HCl_{(g)}$, 5 °C, 24 h, water, 5 °C, 2 d.

The addition of a nucleophile to chiral aldehydes, such as **3.16** (example step v, scheme 3.6), can occur with 1,2-asymmetric induction to give an excess of one of the two possible epimeric products.[109] Two strategies have been developed to exert stereochemical control in such reactions (scheme 3.7): 1) The use of a Lewis acid to

form an intermediate chelate, which reacts with a bulky nucleophile, from the least-hindered face (chelation control); 2) The stereoselective attack of a bulky nucleophile according to electronic and steric factors in the presence of non-chelating reagents (non-chelation control according to Cram's rule[110]). Generally the two methods lead to the opposite diastereoisomer product.



Chelation controlled attack
on the least hindered (back) face



Non-chelation controlled attack
(Newman projection of Cram model)

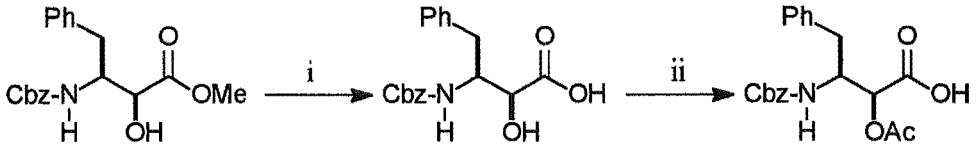
Scheme 3.7: Stereoselective control of nucleophilic addition to chiral aldehydes.

Herranz *et al.*[106] investigated the chelation and non-chelation controlled attack of trimethylsilylcyanide (TMSCN) on Cbz-*D*-phenylalaninal (the enantiomer of **3.16**). As expected, they found that under a chelation controlled attack, the *threo* diastereoisomer product (the enantiomer of **3.20**) was almost exclusively formed. However, under conditions known to favour non-chelation control, an equal mixture of the *threo* and *erythro* (the enantiomer of **3.19**) diastereoisomer products was obtained. The steric bulk of the amine protecting group is important in affecting non-chelation control.[111] The bulkier *N,N*-dibenzylamino aldehydes do form, almost exclusively, non-chelation controlled adducts.[112]

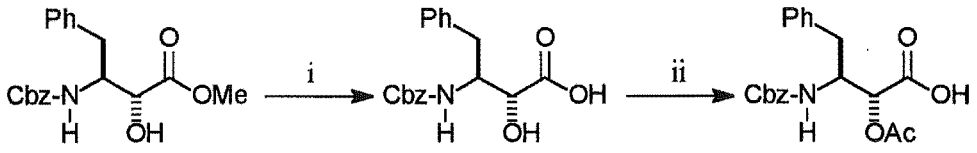
With this in mind it was anticipated that a chelation controlled addition of TMSCN to **3.16** would give a (*2R,3S*)-cyanohydrin adduct with the required configuration found in the target *cis*-hydroxyethylamine isostere **2.12**. However, possibly due to the method of *in situ* preparation of the TMSCN reagent used, equal mixtures of non-silylated cyanohydrin adducts **3.18** were produced. Therefore, in a modified procedure, the aldehyde **3.16** was reacted directly with potassium cyanide in ethyl acetate-water to give a 1:1 mixture (by ^{1}H NMR) of the cyanohydrin epimers **3.18** in 76% combined yield.

The reaction of **3.18** in a solution of methanol-ether, saturated with dry gaseous hydrogen chloride, gave imidate hydrochloride intermediates which were hydrolyzed *in situ* to give a mixture of **3.19** and **3.20** in 59% combined yield (scheme 3.6, step vi). Purification of the mixture by flash chromatography gave pure samples of **3.19** and **3.20**. In an alternative procedure,[113] the cyanohydrin mixture **3.18** was hydrolyzed under harsher conditions (refluxing in aqueous 6M HCl) to give an epimeric mixture of (*2RS,3S*)-3-amino-2-hydroxy-4-phenylbutanoic acid (*N* and *C* deprotected **3.19** and **3.20**). This procedure was not pursued further due to the low yield of product and the fact that amine deprotection was not desired.

The methyl esters **3.19** and **3.20** were identified by comparison of ^{1}H NMR, mp and optical rotation data with that of the enantiomeric literature compounds[106] derived from Cbz-*D*-Phe.



3.19 **3.21** **3.22**

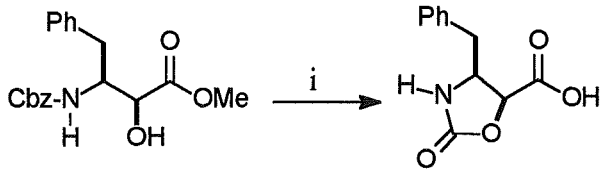


3.20 **3.23** **3.24**

Scheme 3.8: **i**, 1.4 equiv NaOH, MeOH, water, rt, 2 h; **ii**, Ac_2O, pyridine, rt, 18 h.

Hydrolysis of **3.19** (scheme 3.8), with 1.4 equivalents of sodium hydroxide (step i) in methanol-water, for 2 hours, gave the acid **3.21** in 88% yield. The hydroxyl group of **3.21** was then protected, by reaction with acetic anhydride in pyridine (step

ii), to give the acetate **3.22** in quantitative yield. A similar sequence using **3.20** gave the acid **3.23** (88%) and then the acetate **3.24** (quantitative).



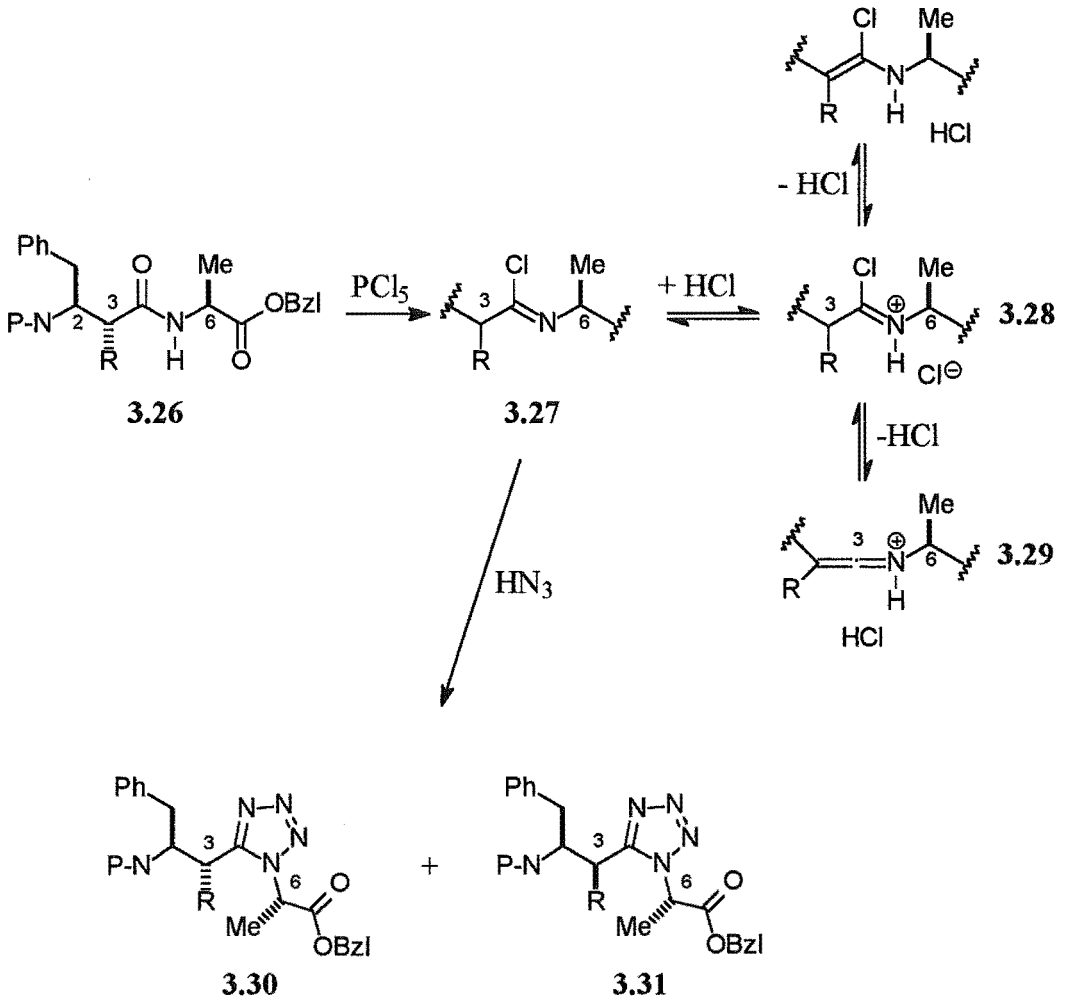
3.19 **3.25**

Scheme 3.9: **i**, 2 equiv K_2CO_3, MeOH, water, rt, 18 h.

Hydrolysis of **3.19** with 2 equivalents of potassium carbonate (scheme 3.9) in methanol-water for 18 hours gave the cyclic *cis*-5-oxazolidinecarboxylic acid **3.25**. The formation of *cis*-**3.25** confirmed the (*2S,3S*)-configuration of **3.19**.

The coupling of the β-amino acids **3.7**, **3.11**, **3.21/3.23**, **3.22, 3.24** and **3.25** with *L*-alanine benzyl ester (schemes 3.12, 3.13, 3.15 and 3.16) using the standard peptide coupling reagents DCC/1-hydroxybenzotriazole (HOBT)[114] or BOP,[115] gave the corresponding amides **3.32**, **3.34**, **3.36/3.37**, **3.39, 3.41,** and **3.43** in 44% to 61% yield, ready for conversion to the corresponding tetrazoles.

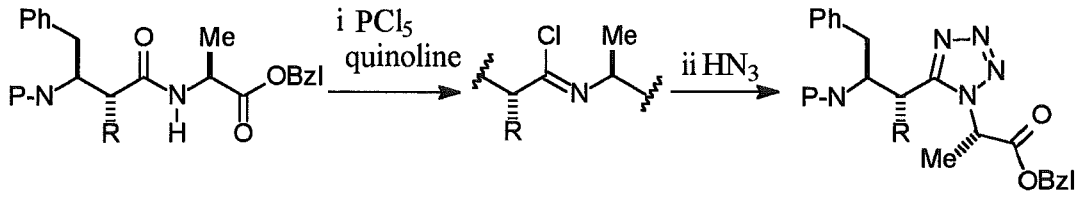
Conversion of a Dipeptide to a Tetrazole



Scheme 3.10: Acid-catalyzed epimerization during tetrazole formation.

The conversion of a protected dipeptide **3.26** into a tetrazole **3.30**, with retention of configuration (scheme 3.11), requires special experimental conditions. The use of PCl_5 to convert the amide bond to the imidoyl chloride **3.27** leads to acid-catalyzed epimerization at C3 by the proposed mechanism shown in scheme 3.10. A reversible elimination of the 3-H of **3.28** to form the dipeptide ketene imine.HCl **3.29**, leads to a scrambling of the configuration. Proton 3-H is more acidic in the hydrochloride **3.28** than in **3.27**, and therefore the simple addition of an organic base, such as quinoline, to the reaction with PCl_5 when generating **3.27**, is effective in

preventing most of the acid-catalyzed epimerization (scheme 3.11). Quinoline is more readily protonated than the intermediate **3.27**.

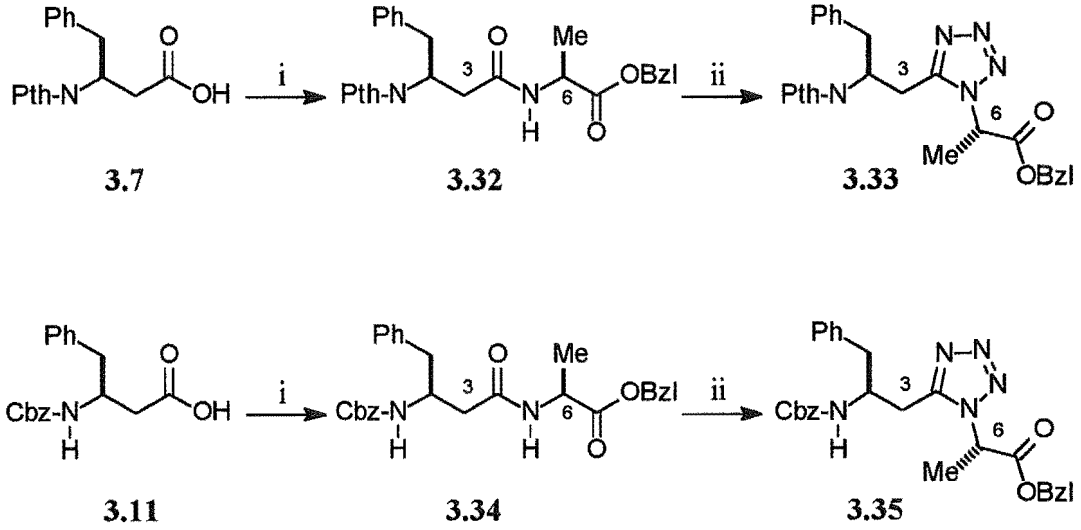


3.26 **3.27** **3.30**

Scheme 3.11: Tetrazole formation with retention of configuration.

The resulting tetrazole **3.30** is quite sensitive to base with both the C3 and C6 centers epimerizing under mildly basic conditions. Therefore, the choice of acidolytic protecting groups is crucial to maintaining chiral integrity. Zabrocki *et al.*[76] preferred the use of Cbz for *N*-terminal amino protection and benzyl ester for *C*-terminal carboxyl protection, with the preferential removal of Cbz, by reaction with HBr in acetic acid, providing a synthetic route to a wide variety of chain-extended tetrazole isosteres. Studies by Yu and Johnson,[116] on the conversion of normal dipeptides to tetrazoles, suggested that the yield of the tetrazole-forming reaction is dependent on the choice of protecting groups and also the amino acid sequence. Dipeptides with bulky amino acid sidechains gave low yields of tetrazole, very probably due to unfavourable *cis*-1,4 steric interactions in the transition state leading to the tetrazole. Cbz and Pth *N*-terminal protecting groups were preferred. Boteju and Hruby[117] used a modified procedure to prepare tetrazoles containing bulky tryptophan sidechains, involving longer reaction times in both steps i and ii (scheme 3.11) and the addition of an extra portion of PCl_5 in the formation of the imidoyl chloride (step i). Longer reaction times were reported to obtain good yields, while shorter reaction times resulted in the recovery of higher percentages of the starting dipeptide. They also observed that the *C*-terminal protecting group had a considerable effect on the overall yield of tetrazole. The use of methyl ester over benzyl ester at the *C*-terminal gave drastic reductions in yield, perhaps surprisingly considering that OMe is smaller than OBzl.

The dipeptide **3.32** was stirred with PCl_5 (1 equivalent) in benzene (scheme 3.12). After 45 minutes, an additional amount of PCl_5 (0.2 equivalents) was added to the reaction mixture. A total reaction time of 1.5 hours was allowed for complete conversion to the imidoyl chloride intermediate. A benzene solution of hydrazoic acid was then added and stirring was continued for 48 hours to give a mixture of the tetrazole **3.33** (51%) and the unreacted dipeptide **3.32** (30%). A similar procedure using the dipeptide **3.34** gave a mixture of the tetrazole **3.35** (47%) and unreacted **3.32** (34%) (scheme 3.12).



3.7 **3.32** **3.33**

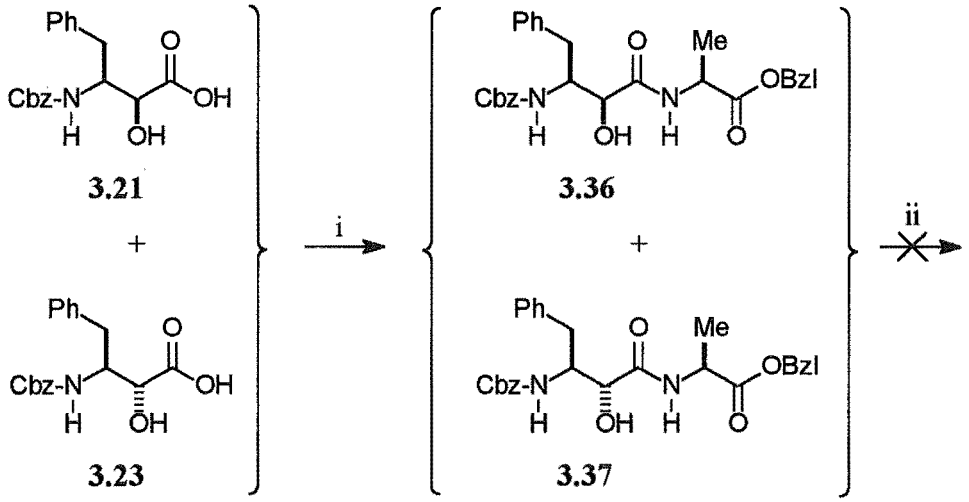
3.11 **3.34** **3.35**

Scheme 3.12: **i**, *L*-Ala-OBzl.HCl, DCC, HOBT, Et_3N, CH_2Cl_2, rt, 18 h; **ii**, PCl_5, benzene, 1.5 h, HN_3, benzene, rt, 2 d.

Since the C3 centers of **3.32** and **3.34** were unsubstituted the addition of quinoline was not required. No epimerization was observed, under these conditions, at the C6 stereocenters of the products **3.33** and **3.35**.

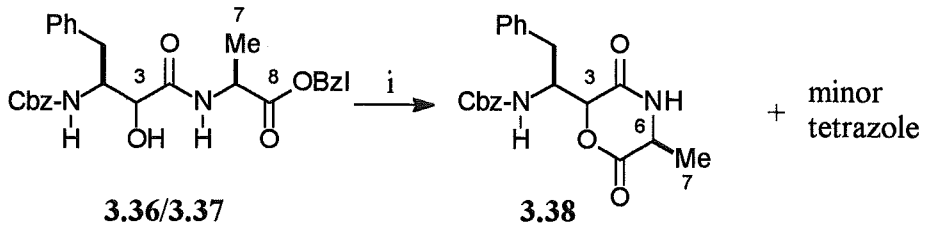
A general procedure for tetrazole formation, modified from above, was followed to convert the dipeptides with a stereocenter at C3. The dipeptide (**3.36/3.37**, **3.39**, **3.41** or **3.43**, schemes 3.13, 3.15 or 3.16) was added slowly to a stirred chloroform solution of PCl_5 (1 equivalent) and quinoline (2 equivalents). After 1 hour, an additional amount of PCl_5 (0.2 equivalents) was added to the reaction mixture. A total reaction time of 3.5 hours was allowed for complete conversion to

the imidoyl chloride intermediate. A benzene solution of hydrazoic acid was then added, as above, and stirring continued for 48 hours to give **3.40**, **3.42** and **3.44** (schemes 3.15 and 3.16)



Scheme 3.13: **i**, *L*-Ala-OBzl.HCl, DCC, HOBT, Et_3N, CH_2Cl_2, rt, 18 h; **ii**, PCl_5, quinoline, $CHCl_3$, rt, 3.5 h, HN_3, benzene, 2 d.

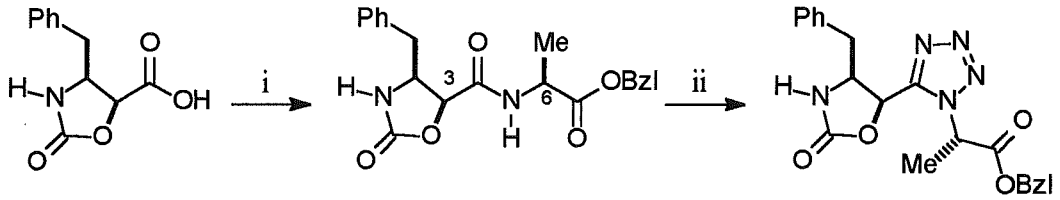
In an initial investigation of the effect of the C3-hydroxyl group on tetrazole formation, a partially separated mixture of **3.36** and **3.37** (4:1 by ^1H NMR) was reacted according to the general procedure above. Two products were observed by ^1H NMR. The major product (85% by ^1H NMR) was a mixture of epimers (7:3 by ^1H NMR), thought to be the amide **3.38** (scheme 3.14). High resolution mass spectroscopy indicated the product had a formula $C_{21}H_{22}N_2O_5$ (M^+ 382.1529), identical to that of the cyclic dipeptide **3.39** (scheme 3.15). However, the ^1H NMR data showed differences with **3.39**. The chemical shifts of the signals, 6-H (4.34 ppm) and 3-H (4.67 ppm) in **3.38**, compared with those of **3.39** (4.70 ppm and 5.10 ppm, respectively),[118] suggested that cyclization had occurred from the C3-hydroxyl group to the C8-carbonyl. This is consistent with the mass spectroscopy. A small yield (15% by ^1H NMR) of an unidentified tetrazole compound was observed by the appearance of a characteristic resonance for the 7-H_3 protons at 1.79 ppm, compared to 1.27 ppm for the dipeptide **3.36** (see chapter four for a discussion of characteristic NMR data).



3.36/3.37 **3.38**

Scheme 3.14: **i**, PCl_5, quinoline, $CHCl_3$, rt, 3.5 h, HN_3, benzene, 2 d.

To investigate further the role of the C3-hydroxyl group, the dipeptide **3.39** (where the hydroxyl group is protected within the oxazolidine ring) was reacted according to the general procedure for tetrazole formation above, to give a mixture (5:3 by ^1H NMR) of the tetrazole **3.40** and the unreacted amide **3.39** (scheme 3.15).



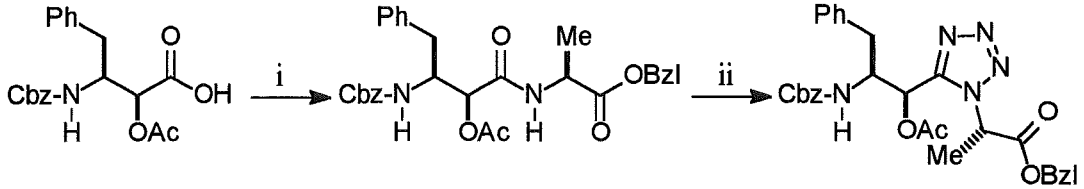
3.25 **3.39** **3.40**

Scheme 3.15: **i**, *L*-Ala-OBzl.HCl, DCC, HOBT, Et_3N, CH_2Cl_2, rt, 18 h; **ii**, PCl_5, quinoline, $CHCl_3$, rt, 3.5 h, HN_3, benzene, 2 d.

Therefore, it was proposed that a suitable protecting group for the C3-hydroxyl function was needed during tetrazole formation. Similarly, in the studies of Boteju and Hruby,[117] protection of the indole nitrogen of the tryptophan side chain was required to prevent low yields (< 5-10%) of tetrazoles. The hydroxyl protecting group should be small and able to be added and removed in high yield. A bulky protecting group may lead to unfavorable steric interactions in the transition state leading to the tetrazole. The tetrazole ring forces a *cis* configuration which increases steric interactions between the C3 and C6 centers and their substituents. In addition, the protecting group should be able to be removed under non-basic conditions due to the base sensitive nature of the tetrazole to epimerization. In separate reactions, the hydroxyl functions of **3.21** and **3.23** were quantitatively protected as the acetates **3.22** and **3.24**

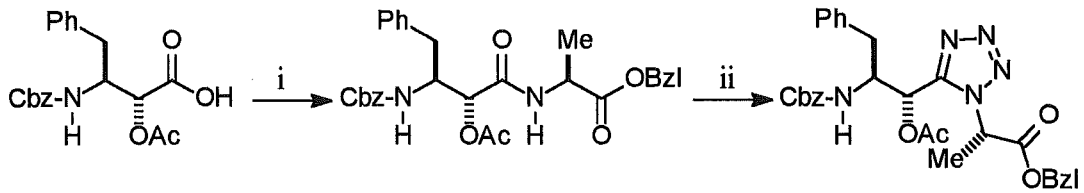
(scheme 3.8, step ii), by reaction with acetic anhydride in pyridine, and used to prepare the dipeptides **3.41** and **3.43**, respectively (scheme 3.16). The acetate group is not an ideal hydroxyl-protecting group for this system however, as it is traditionally removed under mildly basic conditions, which leads to some epimerization of the tetrazoles.

The dipeptide **3.41** was reacted according to the general procedure for tetrazole formation above, to give a mixture of the tetrazoles **3.42** and **3.44** (63%, 17:3 by ^{1}H NMR) and unreacted **3.41** (14%) (scheme 3.16). A similar procedure using **3.43** gave a mixture of the tetrazoles **3.44** and **3.42** (63%, 4:1 by ^{1}H NMR) and unreacted **3.43** (32%) (scheme 3.16). The tetrazoles **3.42** and **3.44** were purified by recrystallization or flash chromatography.



3.22 **3.41** **3.42**

+ minor **3.44**

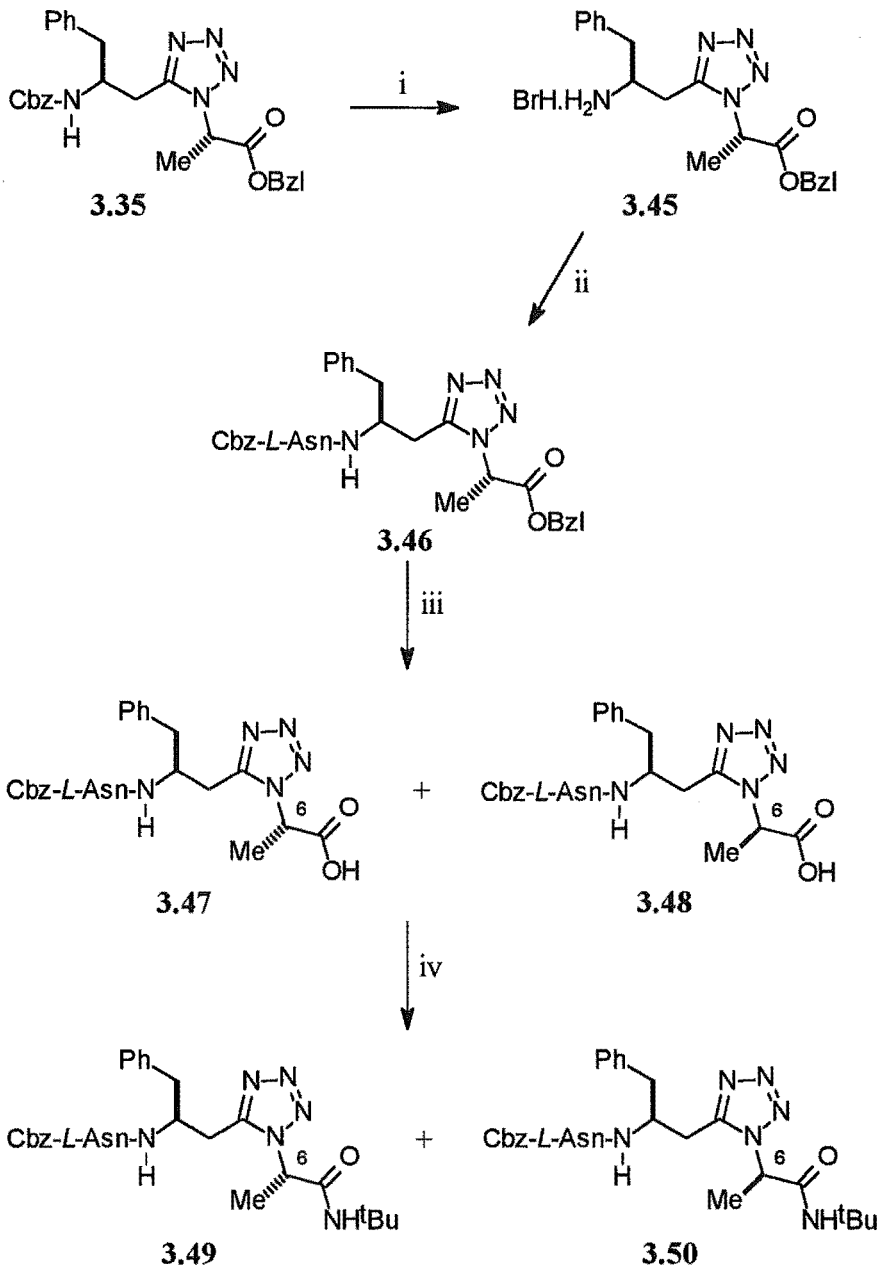


3.24 **3.43** **3.44**

+ minor **3.42**

Scheme 3.16: **i**, *L*-Ala-OBzl.HCl, BOP, Et_3N, CH_2Cl_2; **ii**, PCl_5, quinoline, $CHCl_3$, rt, 3.5 h, HN_3, benzene, 2 d.

Extension in the *N* and *C* Directions



Scheme 3.17: **i**, 50% HBr-acetic acid, rt, 20 min; **ii**, Cbz-*L*-Asn, DCC, HOBT, Et_3N, CH_2Cl_2, rt, 18 h; **iii**, KOH, MeOH, water, rt, 18 h; **iv** $^tBuNH_2$, BOP, Et_3N, CH_2Cl_2, rt, 18 h.

Preferential removal of the Cbz *N*-terminal protecting group in the presence of the benzyl ester *C*-terminal protecting group with HBr in acetic acid, enabled extension

of the tetrazole isosteres by coupling to the free *N*-terminals. Hydrogenation of the benzyl ester in the presence of palladium on carbon catalyst enabled subsequent coupling to the free *C*-terminal.

The tetrazole **3.35** was treated with 50% HBr in acetic acid for twenty minutes to give the amine hydrobromide **3.45** in 84% yield, which was then coupled with *N*-benzyloxycarbonyl-*L*-asparagine (Cbz-*L*-Asn) using DCC/HOBT to give **3.46** in 76% yield (scheme 3.17). Hydrolysis of the benzyl ester **3.46** with 1 equivalent of potassium hydroxide in methanol-water for 18 hours gave an epimeric mixture of **3.47** and **3.48** (17:3 by ^{1}H NMR), where epimerization had occurred at the C6 stereocenter. These mildly basic conditions appeared to catalyze epimerization slowly over time, therefore the more suitable hydrogenation procedure for removal of benzyl ester was used in subsequent deprotections. The reaction of **3.47** and **3.48** with two equivalents of *tert*-butylamine, two equivalents of triethylamine and BOP for 18 hours gave an epimeric mixture of the *tert*-butyl amides **3.49** and **3.50** (3:2 by ^{1}H NMR) in 16% yield (scheme 3.17). The coupling procedure caused further epimerization at the C6 stereocenter, probably due to the presence of two organic bases and the long reaction time. Reverse phase analytical HPLC confirmed the presence of two epimers (figure 3.1). The C6 configurations of **3.49** and **3.50** were assigned on the basis of their NMR data (for a discussion see chapter four).

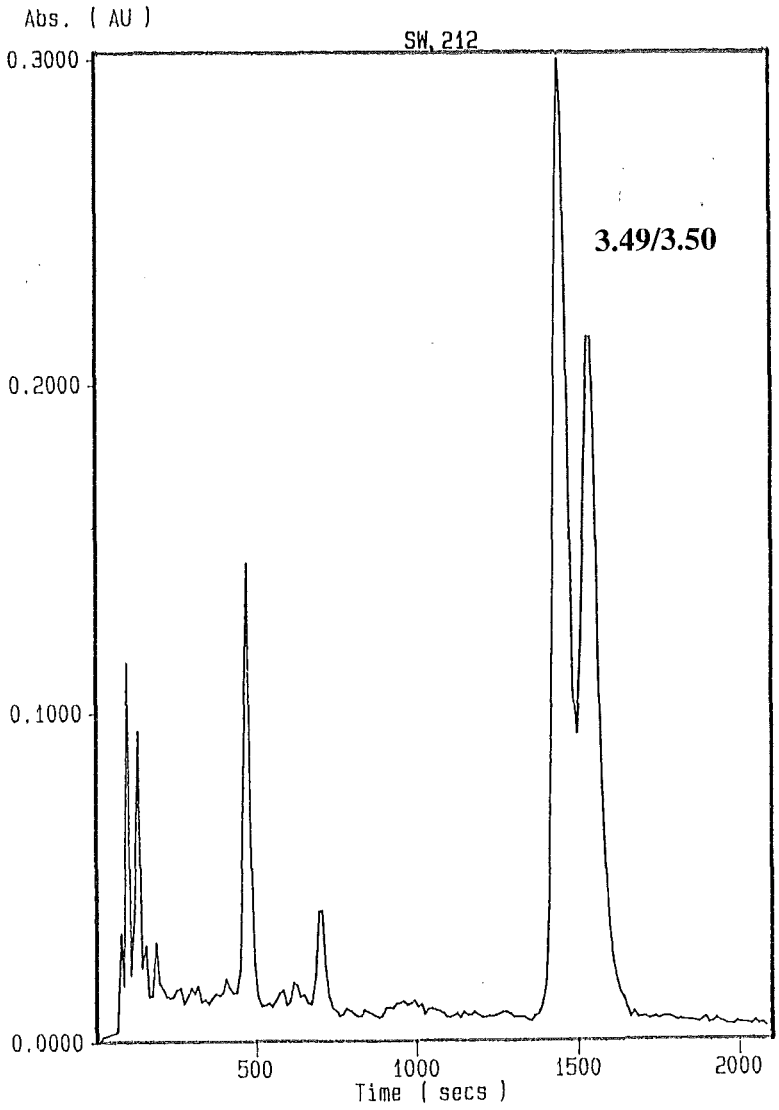
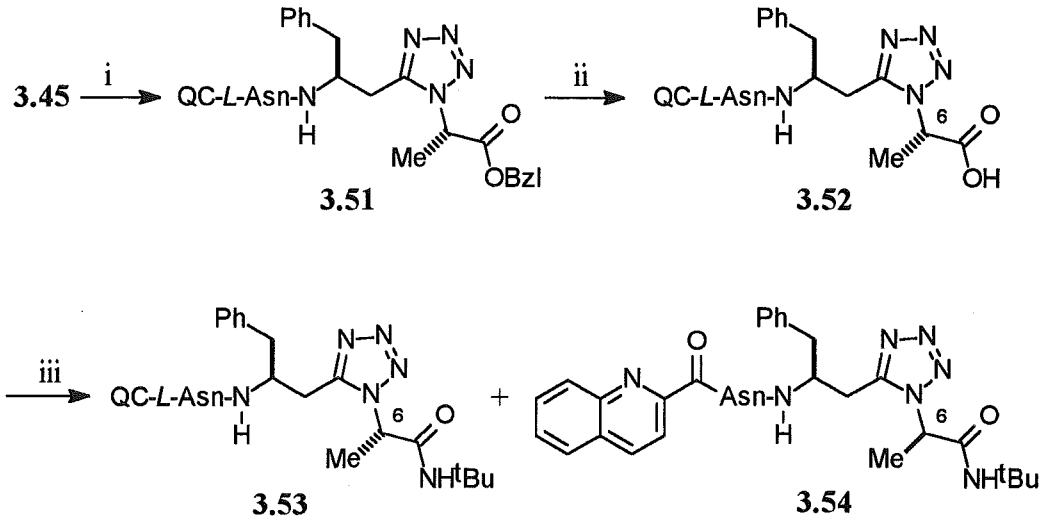


Figure 3.1: HPLC trace of a mixture of **3.49** and **3.50**.



Scheme 3.18: **i**, QC-*L*-Asn, BOP, Et_3N, CH_2Cl_2, DMF, rt, 2 d; **ii**, H_2, 10% Pd/C, acetic acid, EtOH, rt, 18 h; **iii**, $^tBuNH_2$, BOP, Et_3N, CH_2Cl_2, rt, 18 h.

Further amine hydrobromide **3.45** was coupled with *N*-(2-quinolinylcarbonyl)-*L*-asparagine (QC-*L*-Asn) using BOP to give **3.51** in 60% yield (scheme 3.18). QC-*L*-Asn was prepared[119] by a coupling of *L*-asparagine *tert*-butyl ester with quinaldic acid using BOP to give QC-*L*-Asn-O^{t}Bu, which was then converted to the free acid by reaction with trifluoroacetic acid. A hydrogenation of the benzyl ester **3.51** with a catalytic amount of 10% Pd/C gave the acid **3.52** in 79% yield. This was then coupled with *tert*-butylamine using BOP, to give an epimeric mixture of the *tert*-butyl amides **3.53** and **3.54** (1:1 by ^{1}H NMR) in 25% combined yield (scheme 3.18). The epimers **3.53** and **3.54** were separated by reverse phase HPLC (figure 3.2). Coupling to the *C*-terminal had caused epimerization at C6, as observed above. In an attempt to prevent epimerization, coupling procedures using only 1 equivalent of the organic bases *tert*-butylamine and triethylamine, over shorter reaction times, were tried. However, these procedures gave back unreacted free acid and negligible yields of *tert*-butyl amide. The C6 configurations of **3.53** and **3.54** were assigned on the basis of their NMR data (for a discussion see chapter four).

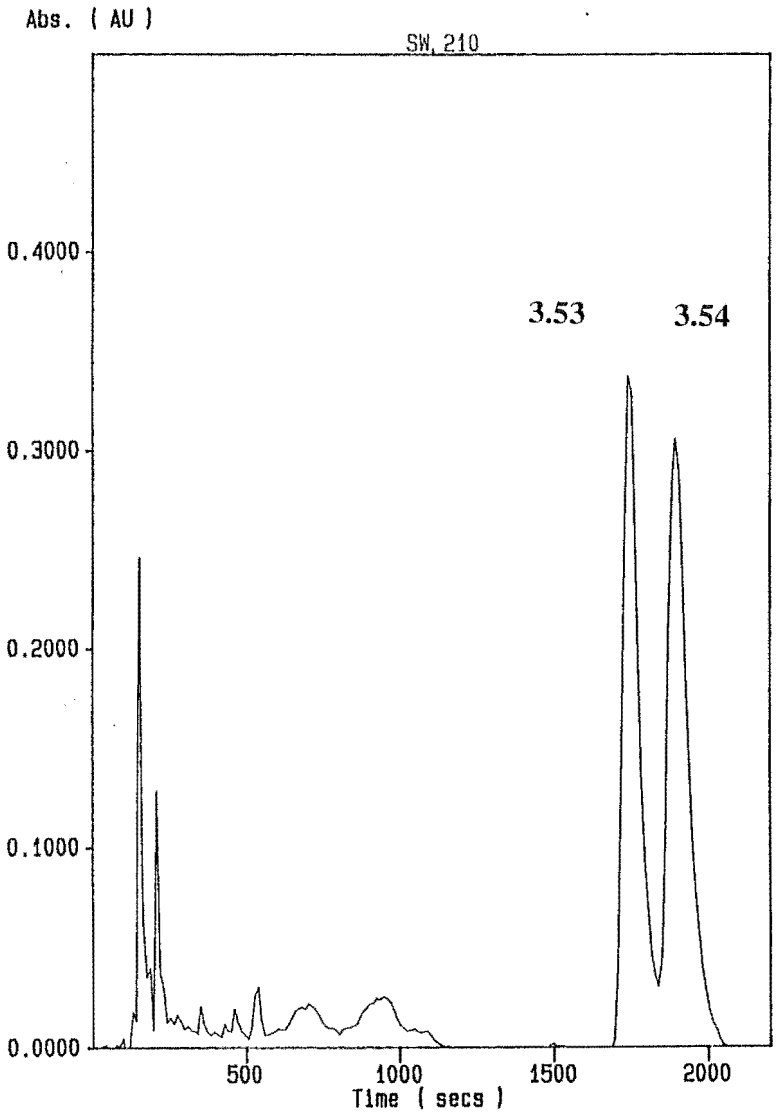
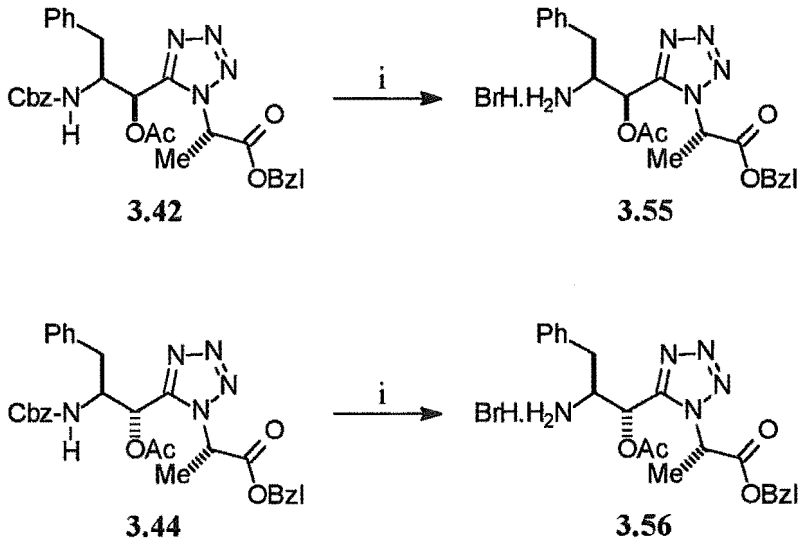


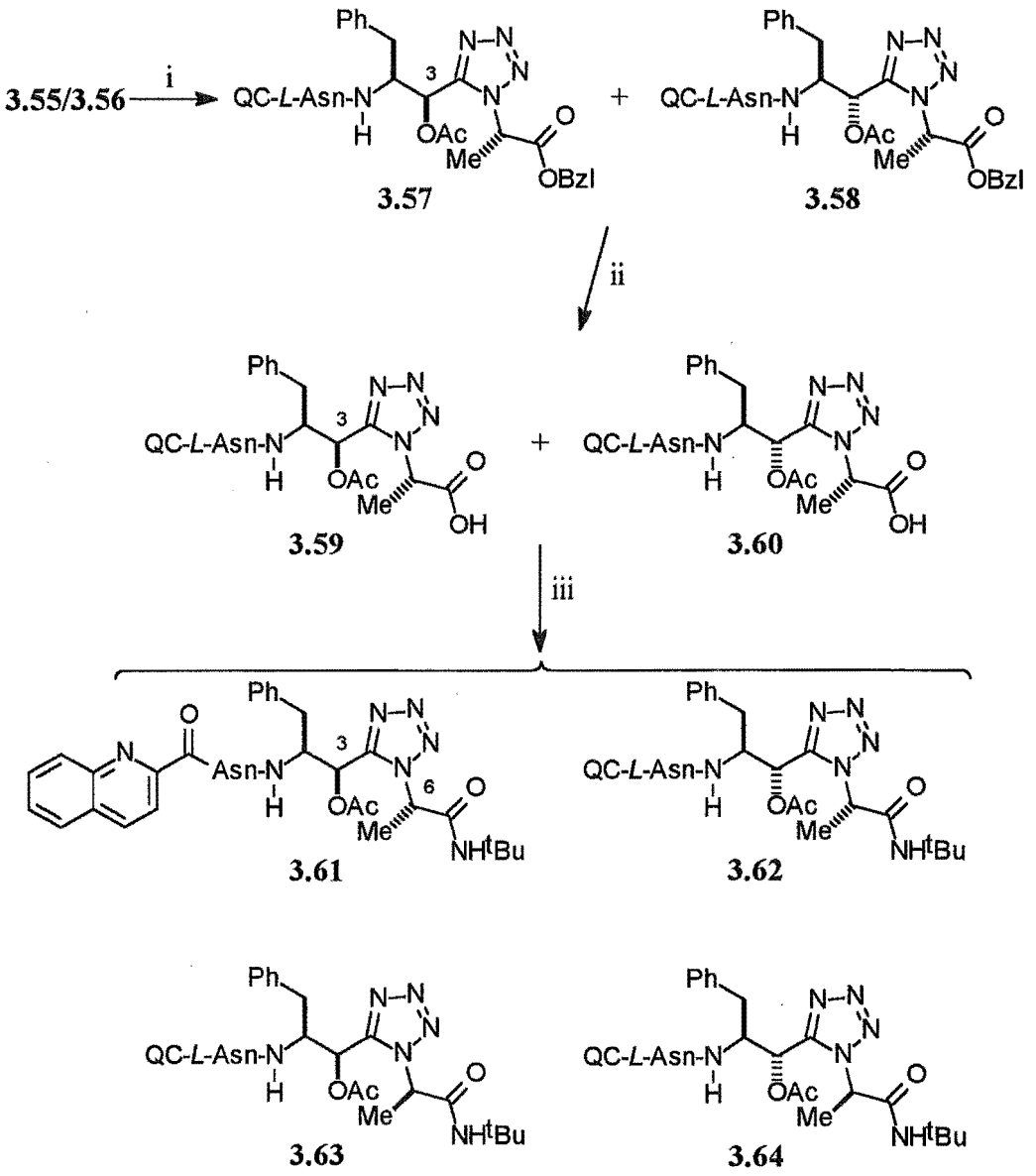
Figure 3.2: HPLC trace of a mixture of **3.53** (1st peak) and **3.54** (2nd peak).

The tetrazole **3.42** was deprotected by reaction with 50% HBr in acetic acid to give the amine hydrobromide **3.55** in 84% yield. A similar procedure using **3.44** gave the amine hydrobromide **3.56** in 89% yield (scheme 3.19).



Scheme 3.19: **i**, HBr, acetic acid, rt, 20 min.

A mixture of **3.55** and **3.56** (3:2 by ^{1}H NMR) was coupled with QC-*L*-Asn using BOP to give a mixture of **3.57** and **3.58**, which was used without further purification (scheme 3.20). Hydrogenation of the mixture gave the free acids **3.59** and **3.60** which were then coupled with 5 equivalents of *tert*-butylamine using BOP, to give a diastereoisomeric mixture of the *tert*-butyl amides **3.61-3.64** (7% yield from **3.42/3.44**). Reverse phase HPLC showed four peaks (figure 3.3), corresponding to the four diastereoisomers resulting from epimerization at the C3 and C6 stereocenters adjacent to the tetrazole ring (scheme 3.20). The C3 and C6 configurations of **3.57-3.64** were assigned on the basis of their NMR data (for a discussion see chapter four).



Scheme 3.20: **i**, QC-*L*-Asn, BOP, Et_3N, CH_2Cl_2, DMF, rt, 2 d; **ii**, H_2, 10% Pd/C, EtOH, rt, 18 h; **iii**, $^tBuNH_2$, BOP, Et_3N, DMF, rt, 18 h.

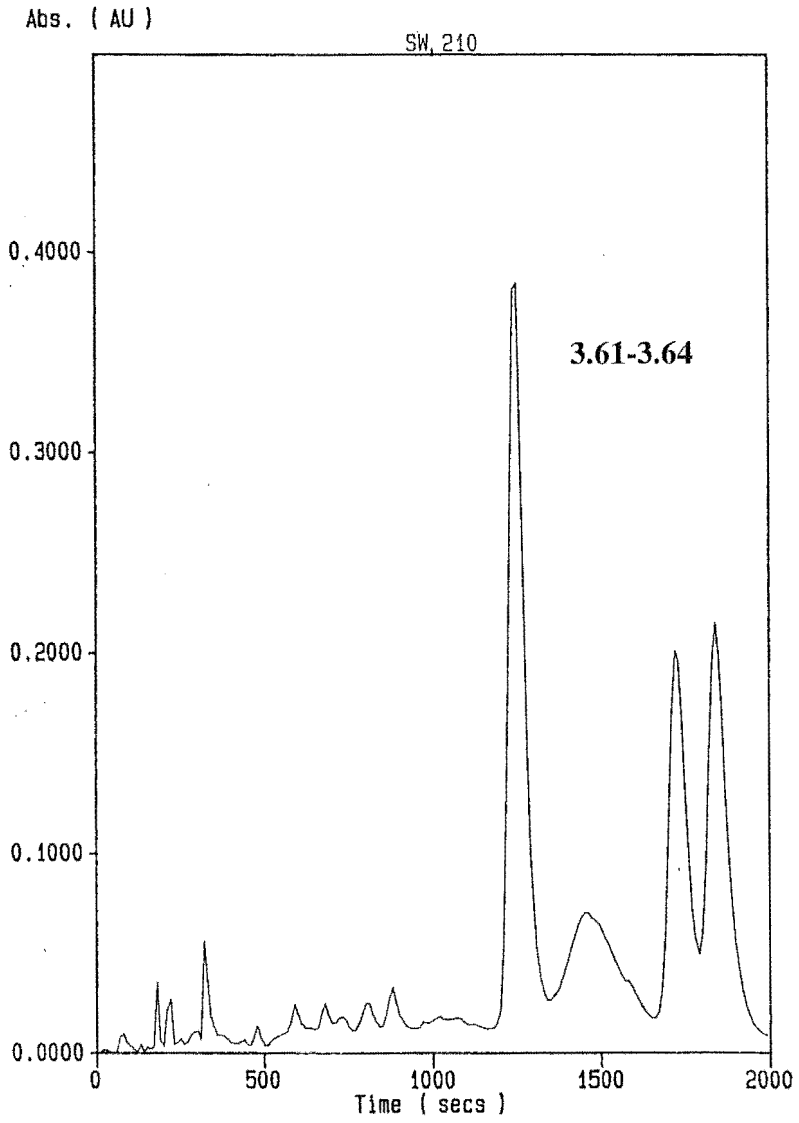
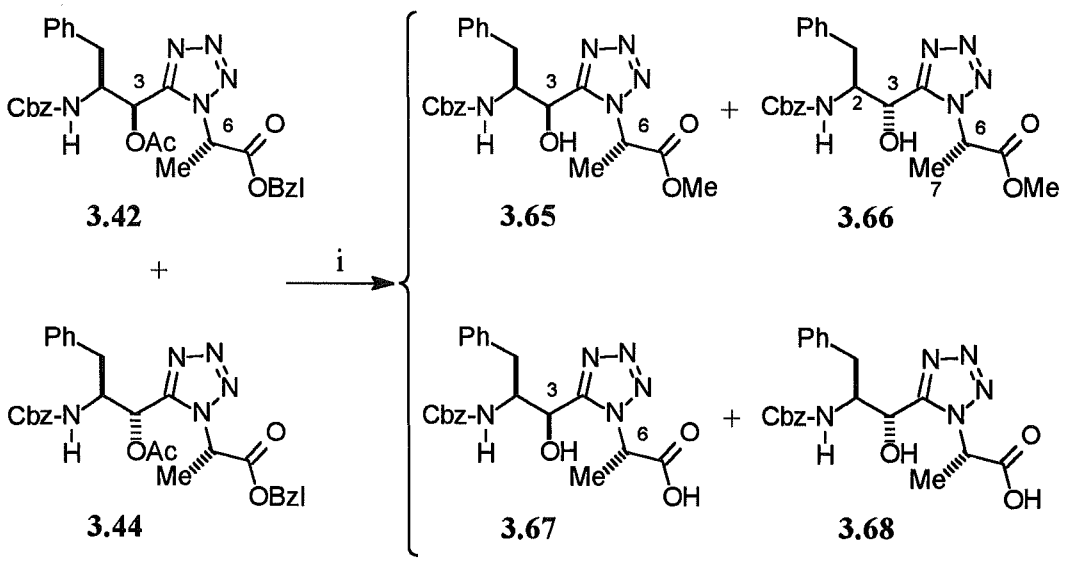


Figure 3.3: HPLC trace of a mixture of **3.61-3.64**.

Experiments investigating the removal of the acetate protecting group were performed on the tetrazoles **3.42** and **3.44** (scheme 3.21). The reaction of a mixture of **3.44** and **3.42** (4:1 by ^{1}H NMR) with 0.2 equivalents of potassium carbonate in methanol-water for 30 minutes gave a mixture of the methyl esters **3.66** and **3.65** (40%, 7:3 by ^{1}H NMR) and the acids **3.68** and **3.67** (29%, 3:1 by ^{1}H NMR); products where both the acetate and benzyl ester protecting groups had been removed. Some epimerization was observed (by ^{1}H NMR) at the C3 stereocenters during the hydrolysis, seen in the distribution of the four products, with an increase in the

amounts of the (*3S*)-epimers **3.65** and **3.67** relative to their corresponding (*3R*)-epimers **3.66** and **3.68**, respectively. Despite epimerization the (*3R*)-compounds **3.66** and **3.68** were still the dominant components, as compared to the starting (*3R*)-epimer **3.44**. Also, no significant epimerization was observed at the C6 center, adjacent to the *C*-terminal. Yu and Johnson[116] however, observed significant epimerization adjacent to the *C*-terminal of the dipeptide derived tetrazole Cbz-*L*-Pro-ψ[CN_4]-*L*-Leu-OMe after a similar hydrolysis of the methyl ester under the harsher basic conditions of 1 equivalent of sodium hydroxide in methanol-water for 1.5 hours.

A similar reaction on a mixture of **3.42** and **3.44** (17:3 by ^{1}H NMR) gave corresponding mixtures of **3.65-3.68** were the (*3S*)-compounds **3.65** and **3.67** were the dominant components. The configurations of **3.65-3.68** were assigned on the basis of the product distributions of the two reactions described, and the known configurations of **3.42** and **3.44**. The methyl esters **3.65** and **3.66** were separated from the acids **3.67** and **3.68** by flash chromatography. The epimers **3.65** and **3.66** were separated from each other by reverse phase HPLC (figure 3.4), and tested for HIV protease inhibition. The ^{1}H NMR spectrum of **3.66** is shown in figure 3.5.



Scheme 3.21: **i**, K_2CO_3, MeOH-water, rt, 30 min.

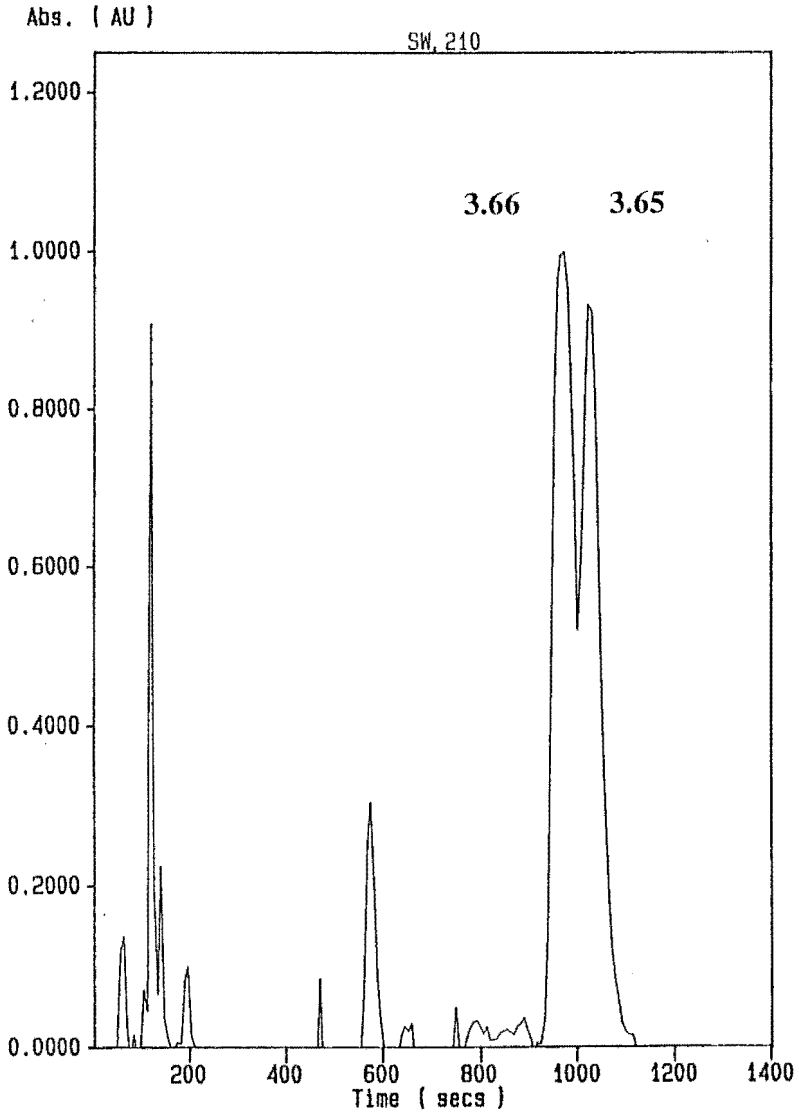


Figure 3.4: HPLC trace of a mixture of **3.66** (1st peak) and **3.65** (2nd peak).

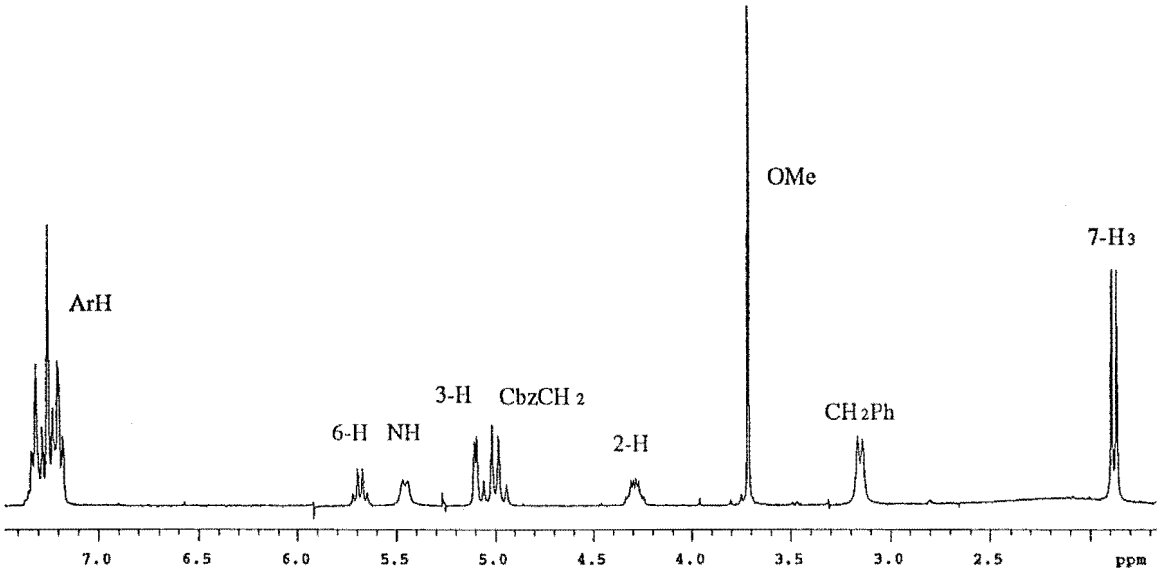
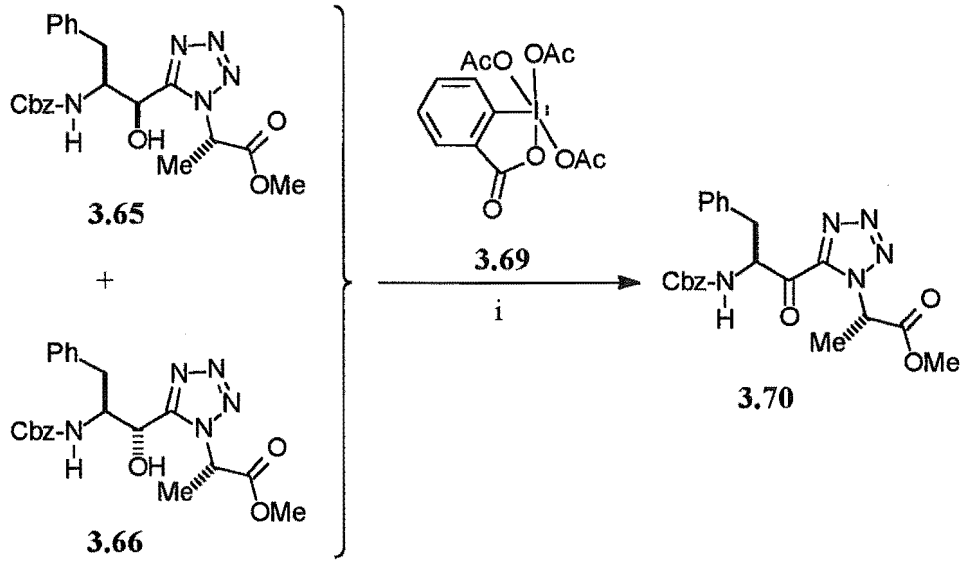


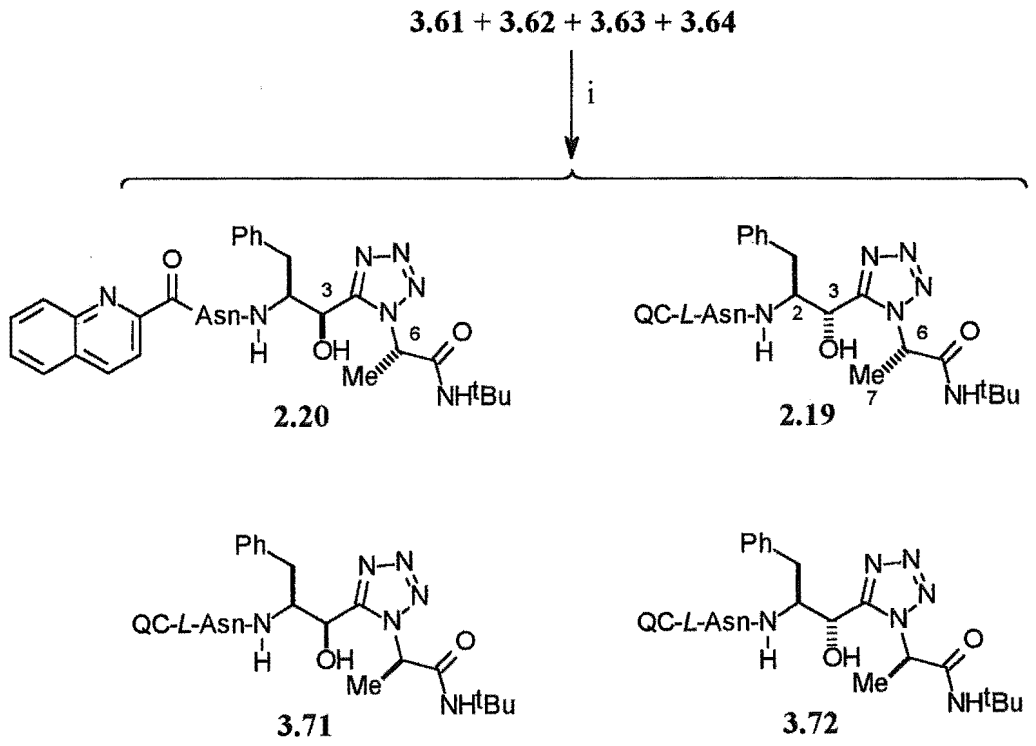
Figure 3.5: ^{1}H NMR spectrum (solvent $CDCl_3$) of **3.66**.

Slee *et al.*[80] described the oxidation of the hydroxyl group of hydroxyamide dipeptide isosteres as a general preparative route to the potent α-keto amide isostere (see figure 2.2). An investigation of the potential expansion of our tetrazole isostere **2.12** to encompass the design principle of the α-keto amide core (compound **2.13**, see chapter 2 for discussion) was carried out on the tetrazoles **3.65** and **3.66**. The reaction of a mixture of **3.65** and **3.66** (1:1 by ^{1}H NMR) with 5 equivalents of Dess-Martin periodinane **3.69** for 18 hours gave the α-keto tetrazole **3.70** (quant) (scheme 3.22). Dess-Martin periodinane[108] is a mild and selective oxidizing agent, widely used for the conversion of primary and secondary alcohols to aldehydes and ketones, respectively, in high yield.



Scheme 3.22: **i**, **3.69**, CH_2Cl_2, rt, 18 h.

A hydrolysis of the diastereoisomeric mixture of acetates **3.61-3.64** (4 mg) with two equivalents of potassium carbonate in methanol-water for 18 hours (scheme 3.23) gave a diastereoisomeric mixture of the alcohols **2.19**, **2.20**, **3.71** and **3.72** (quant), which were separated by reverse phase HPLC (figure 3.7), and tested for HIV protease inhibition. The C3 and C6 configurations of **2.19**, **2.20**, **3.71** and **3.72** were assigned on the basis of their NMR data (for a discussion see chapter four). The ^{1}H NMR spectrum of **2.19** is shown in figure 3.6.



Scheme 3.23: **i**, K_2CO_3, MeOH-water, rt, 18 h.

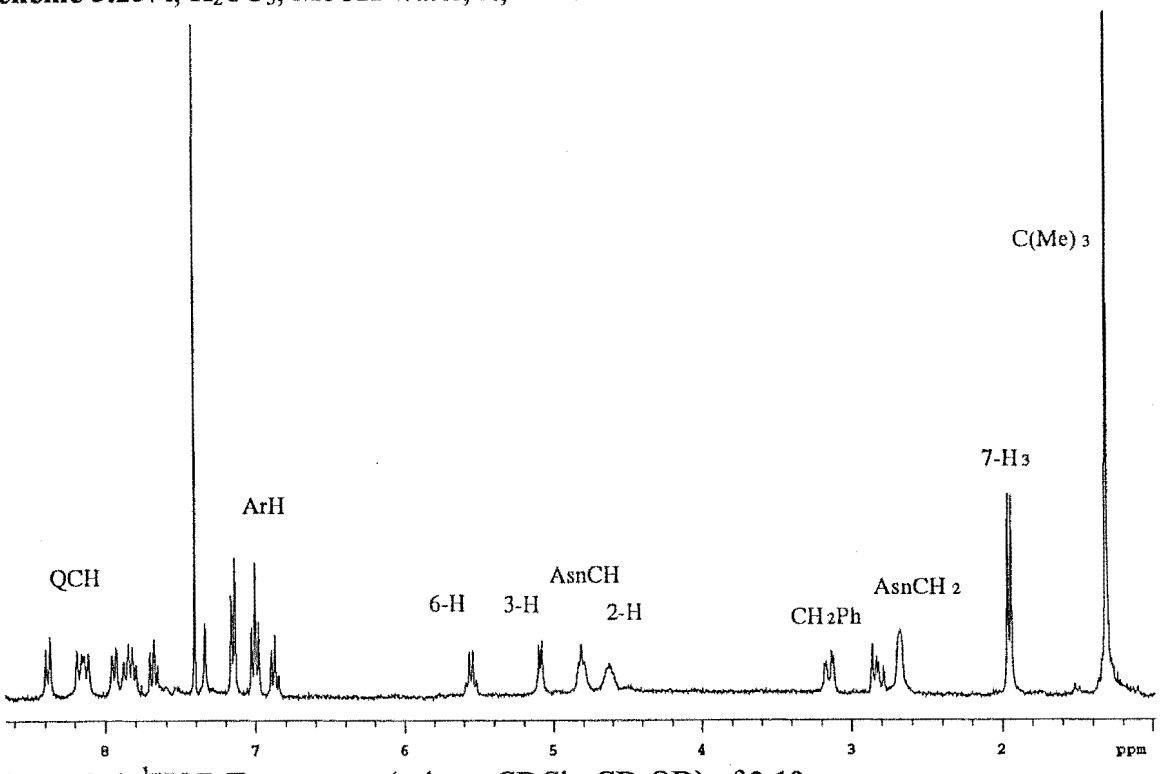


Figure 3.6: ^1H NMR spectrum (solvent $CDCl_3$, CD_3OD) of **2.19**.

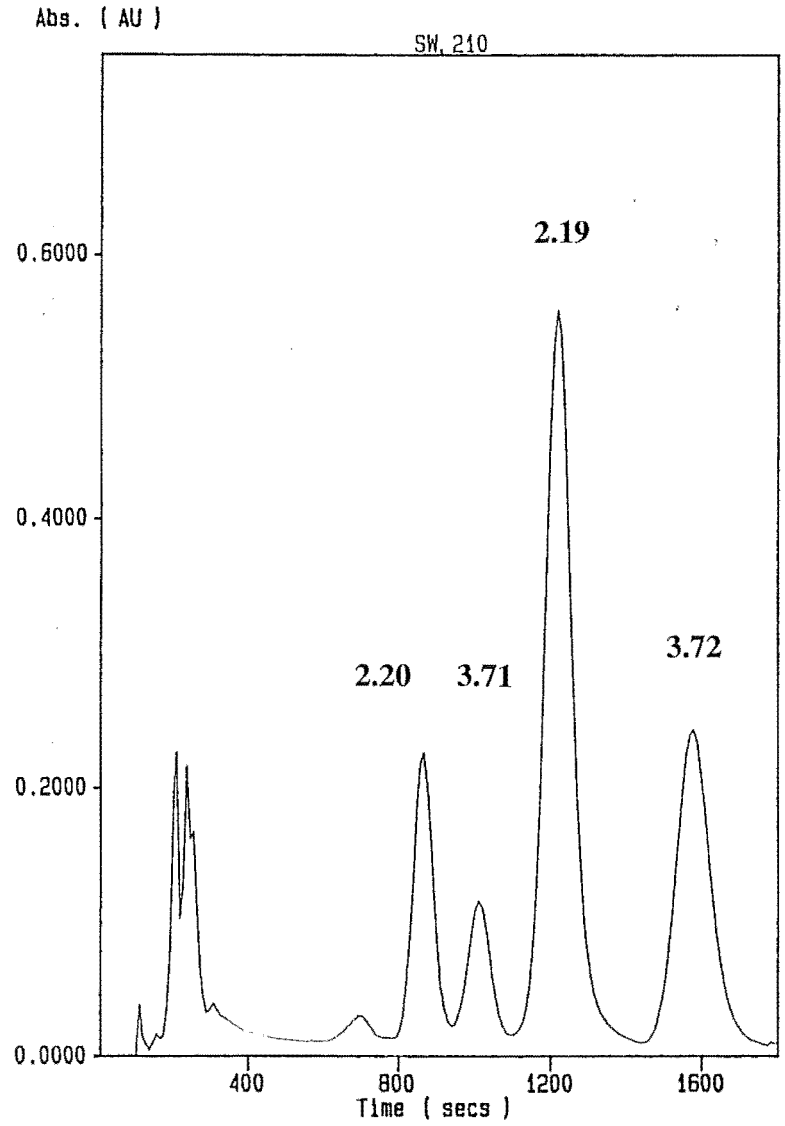


Figure 3.7: HPLC trace of a mixture of **2.20** (1st peak), **3.71** (2nd), **2.19** (3rd), and **3.72** (4th).

Conclusion

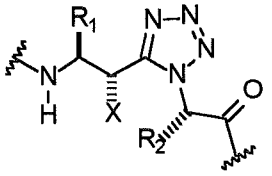
The procedure for the conversion of a dipeptide to a tetrazole was developed and applied successfully to a range of unusual dipeptides, based on the β-amino acids **3.7**, **3.11**, **3.19**, **3.20** and **3.25**. These β-amino acids were themselves prepared from the corresponding α-amino acids. General procedures for the extension of the series of tetrazole dipeptide isosteres in the *N* and *C* directions were developed, and a series of potential HIV protease inhibitors based on the target **2.12** was synthesized. The activity of this series of compounds is discussed in chapter four. Structure **2.12** was also shown to be easily converted to the second target isostere, α-keto tetrazole **2.13**.

Chapter Four

NMR Spectroscopic Analysis and HIV-1 Protease Inhibition Studies of the *cis*-Hydroxyethylamine Isostere

NMR Spectroscopic Studies

The ^{1}H and ^{13}C NMR data of the synthesized tetrazole derivatives, with general structure **2.12**, show some characteristic trends.

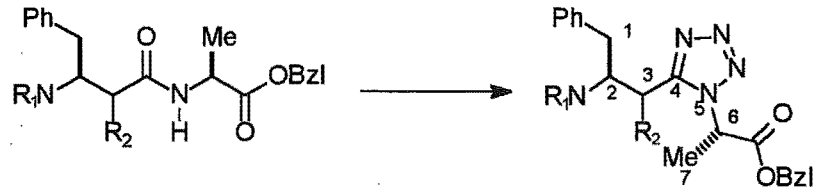


cis-hydroxyethylamine isostere **2.12**

A series of tetrazole derivatives, including **3.33**, **3.35**, **3.40**, **3.42** and **3.44**, was synthesized from the corresponding amides **3.32**, **3.34**, **3.39**, **3.41** and **3.43**, as described in chapter three (see scheme 4.1 for a summary).

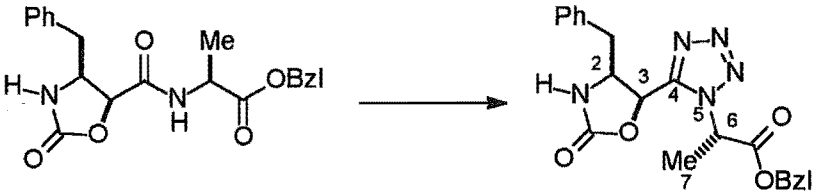
Typically, the tetrazoles are less polar (eluting faster on silica) by comparison to the corresponding starting amides, and display some distinctive differences in their ^{1}H and ^{13}C NMR data. The formation of the tetrazole ring was evidenced, in these reactions, by the disappearance of the amide carbonyl carbon resonance at around 170 ppm and the appearance of a new signal for the tetrazole C4 carbon (non-systematic numbering, see scheme 4.1) in the range 151.22 to 152.76 ppm. It was also observed in the ^{13}C NMR spectra of the tetrazole products, that the resonance for C6 was shifted downfield in the order of 6-8 ppm, to around 56 ppm, compared with that in the corresponding amide. In addition, the resonance for C3 was shifted upfield 8 to 13 ppm. An example is given in figure 4.1, where part of the ^{13}C NMR spectra of the dipeptide **3.43** and the corresponding tetrazole **3.44** are shown for comparison. Similar

differences in chemical shifts were reported by Yu and Johnson[116] in the conversion of normal dipeptides to the corresponding tetrazole derivatives.



3.32 R_1 = Pth, R_2 = H
3.34 R_1 = CbzH, R_2 = H
(*S*)-**3.41** R_1 = CbzH, R_2 = OAc
(*R*)-**3.43** R_1 = CbzH, R_2 = OAc

3.33 R_1 = Pth, R_2 = H
3.35 R_1 = CbzH, R_2 = H
(*S*)-**3.42** R_1 = CbzH, R_2 = OAc
(*R*)-**3.44** R_1 = CbzH, R_2 = OAc



3.39 **3.40**

Scheme 4.1: Preparation of the series of tetrazole derivatives.

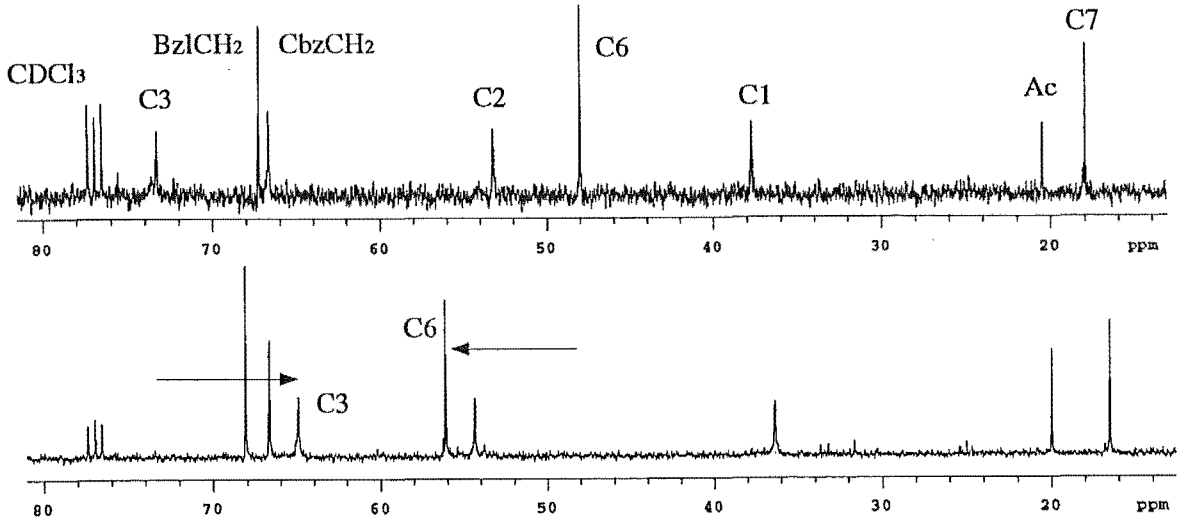


Figure 4.1: Partial ^{13}C NMR spectra (solvent $CDCl_3$) of the dipeptide **3.43** (top) and the corresponding tetrazole **3.44** (bottom), with characteristic resonance shifts shown.

These resonance shifts are presumably associated with differences in the electron density, and steric effects, of an amide and a tetrazole. The lone pair of the tetrazole N5 nitrogen (non systematic numbering, see scheme 4.1) is delocalized throughout the tetrazole ring, thus it is relatively more electron deficient than the nitrogen of the amide bond. On the other hand, the C4 carbon of the tetrazole is more electron rich than the carbonyl carbon of the amide because of the mesomeric effect of the tetrazole ring.

The 6-H and 7-H_3 proton resonances of the tetrazole derivatives were shifted downfield 0.4-1.0 ppm and 0.47-0.61 ppm, respectively, compared with those of the corresponding amides. Likewise, the 3-H resonances were shifted downfield 0.5-1.05 ppm, for both the C3-substituted and non-substituted derivatives. The 2-H resonances of the tetrazoles were also shifted downfield, relatively, however only by 0.15 ppm or less. An example of these shifts is shown in figure 4.2, which shows the ^{1}H NMR spectra of the dipeptide **3.43** and the corresponding tetrazole **3.44**.

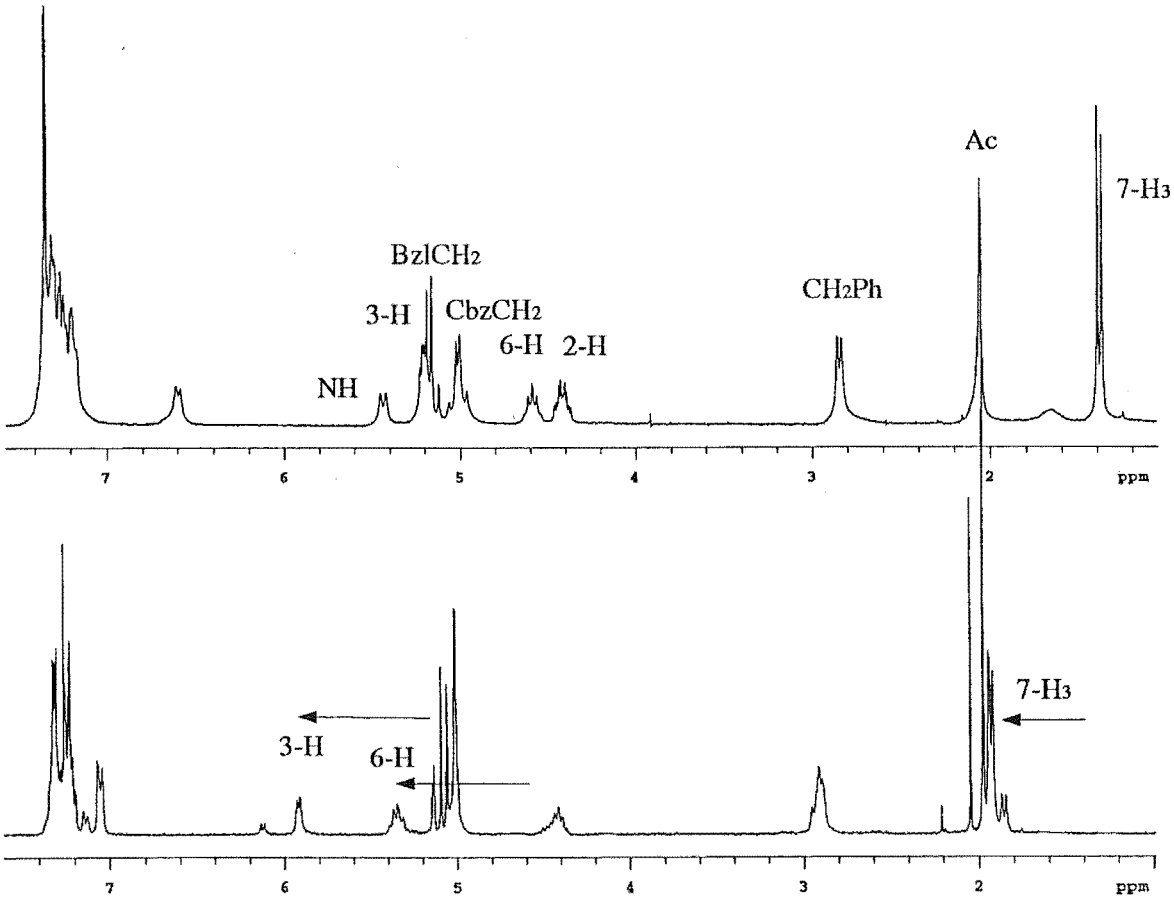
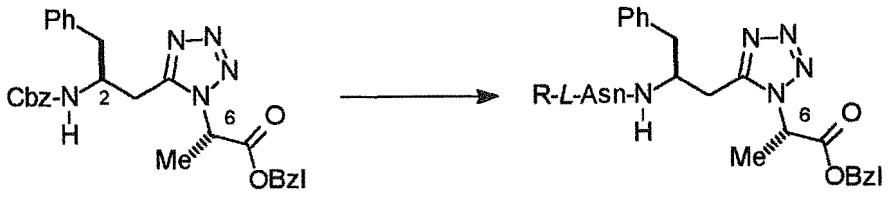


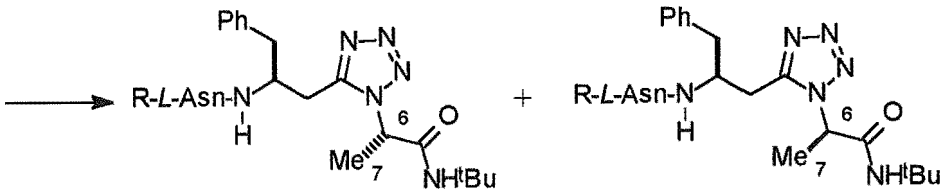
Figure 4.2: ^{1}H NMR spectra (solvent $CDCl_3$) of the dipeptide **3.43** (top) and the corresponding tetrazole **3.44** (bottom) (contains <10% **3.42**).

The tetrazole **3.35** was prepared with a known configuration at C2 and C6. Deprotection and chain extension then gave and derivatives **3.46** and **3.51**, with retention of configuration (scheme 4.2). However, during the preparations of **3.49/3.50** and **3.53/3.54**, epimerization occurred at C6, therefore requiring an assignment of the C6 configurations of these compounds.



3.35

3.46 R = Cbz
3.51 R = QC



3.49 R = Cbz
3.53 R = QC

3.50 R = Cbz
3.54 R = QC

Scheme 4.2: Epimerization at the C6 stereocenter during the preparation of **3.49/3.50** and **3.53/3.54**.

An analysis of the ^{1}H NMR data revealed a trend in the chemical shifts of the 7-H_3 resonances, from which the configurations at the adjacent C6 stereocenters were able to be assigned. This trend was observed throughout the entire series of tetrazole derivatives. Tetrazoles with a (*6S*)-configuration gave 7-H_3 resonances in a characteristic downfield position (1.86 to 1.92 ppm) relative to the corresponding (*6R*)-epimers (1.74 to 1.80 ppm). On this basis, **3.50** and **3.54** were assigned a (*6R*)-configuration with 7-H_3 resonances in upfield positions by 0.09 and 0.18 ppm, respectively, compared to those of **3.49** and **3.53** (table 4.1).

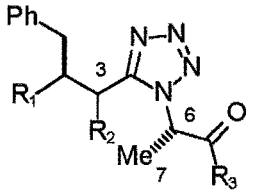


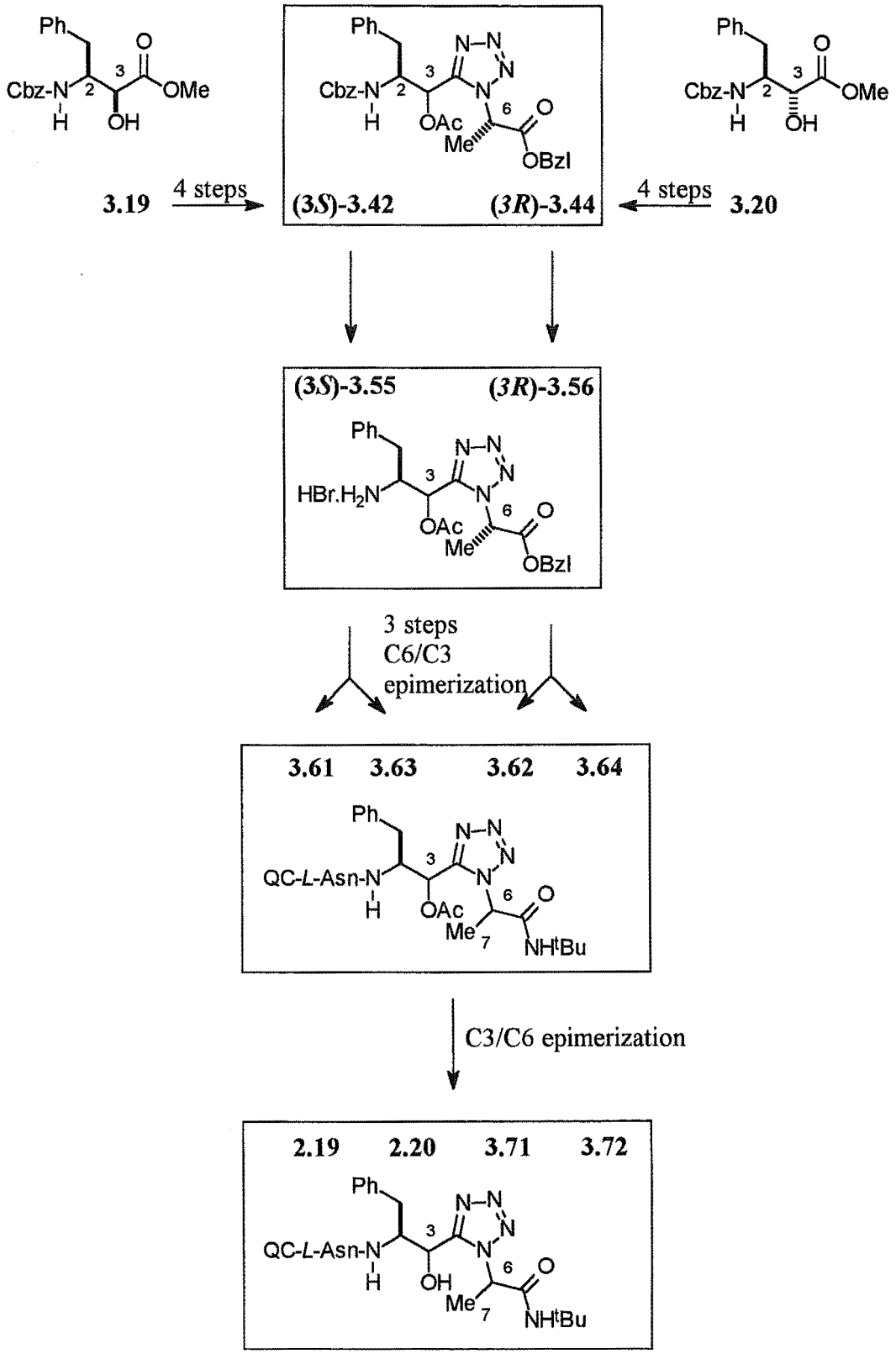
Table 4.1: Characteristic ^{1}H NMR 7-H_3 resonances of the tetrazole derivatives (solvent $CDCl_3$ unless specified).

3.x	R_1	R_2	R_3	C3	C6	δ 7-H_3 (ppm)
3.33	PthN	H	OBzl		*S*	1.86
3.35	Cbz-HN	H	OBzl		*S*	1.88
3.46	Cbz-*L*-Asn-HN	H	OBzl		*S*	1.89[a]
3.49	Cbz-*L*-Asn-HN	H	NH^{t}Bu		*S*	1.87[a]
3.50	Cbz-*L*-Asn-HN	H	NH^{t}Bu		***R***	**1.78**[a]
3.51	QC-*L*-Asn-HN	H	OBzl		*S*	1.88
3.53	QC-*L*-Asn-HN	H	NH^{t}Bu		*S*	1.92[b]
3.54	QC-*L*-Asn-HN	H	NH^{t}Bu		***R***	**1.74**[b]
3.65	Cbz-HN	OH	OMe	*S*	*S*	1.92
3.66	Cbz-HN	OH	OMe	*R*	*S*	1.88

a $CDCl_3$, 1 drop d_6-DMSO

b $CDCl_3$, 2 drops CD_3OD

Scheme 4.2 outlines the preparation of the tetrazoles **2.19**, **2.20**, **3.71** and **3.72** from the β-amino acids **3.19** and **3.20**. Compounds in the sequence up to **3.55** and **3.56**, were prepared separately from the reference compounds **3.19** and **3.20**, with known configurations at C2, C3 and C6. However in the final steps of the reaction sequence, epimerization occurred during the preparation of the derivatives **3.57/3.58**, **3.61-3.64** and **2.19**, **2.20**, **3.71, 3.72**, so that the C3 and C6 configurations of these compounds needed to be assigned. Some characteristic ^{1}H NMR data for the tetrazole derivatives in the reaction sequence (scheme 4.2) is shown in table 4.2, from which the unknown configurations at C3 and C6 were assigned.



Scheme 4.2: Epimerization during the preparation of the epimers **2.19**, **2.20**, **3.71** and **3.72**.

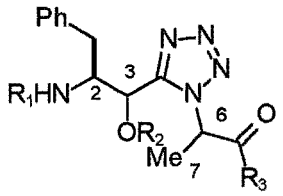


Table 4.2: Characteristic ^{1}H NMR data for tetrazole derivatives leading to **2.19**, **2.20**, **3.71** and **3.72** (solvent $CDCl_3$ unless specified).

3.x	R_1	R_2	R_3	C3	C6	δ 2-H	δ 3-H	δ 6-H	δ 7-H_3
3.42	Cbz	Ac	OBzl	*S*	*S*	4.58	6.09	5.17	1.84
3.44	Cbz	Ac	OBzl	*R*	*S*	4.42	5.92	5.36	1.93
3.55[a]	HBr.H	Ac	OBzl	*S*	*S*	4.30	6.28	5.65	1.89
3.56[a]	HBr.H	Ac	OBzl	*R*	*S*	4.08	5.89	5.71	1.93
3.57	QC-Asn	Ac	OBzl	*S*	*S*	4.6	6.36	5.8	1.92
3.58	QC-Asn	Ac	OBzl	*R*	*S*	*	6.12	*	1.98
3.61	QC-Asn	Ac	NH^{t}Bu	*S*	*S*	4.57-4.68	6.32	5.42	1.97
3.62	QC-Asn	Ac	NH^{t}Bu	*R*	*S*	*	6.24	5.58	2.06
3.63	QC-Asn	Ac	NH^{t}Bu	*S*	*R*	*	6.22	5.27	1.82
3.64	QC-Asn	Ac	NH^{t}Bu	*R*	*R*	*	6.18	5.34	1.85
2.20[b]	QC-Asn	H	NH^{t}Bu	*S*	*S*	4.65	5.12	5.52	1.93
2.19[b]	QC-Asn	H	NH^{t}Bu	*R*	*S*	4.62	5.09	5.55	1.95
3.71[b]	QC-Asn	H	NH^{t}Bu	*S*	*R*	4.61	5.04	5.42	1.80
3.72[b]	QC-Asn	H	NH^{t}Bu	*R*	*R*	4.58	5.00	5.49	1.79

a CD_3OD

b $CDCl_3$, 2 drops CD_3OD

The correlations made above in table 4.1, between the 7-H_3 resonances and C6 configurations of the tetrazole derivatives, were also applied to the series shown in table 4.2. On this basis, the two acetates **3.63** and **3.64**, with upfield 7-H_3 resonances at 1.82 and 1.85 ppm, respectively, were assigned a (*6R*)-configuration. The corresponding (*6S*)-epimers **3.61** and **3.62**, gave 7-H_3 resonances in relatively downfield positions, typically by 0.15 to 0.21 ppm. Similarly, the hydroxyl derivatives **3.71** and **3.72**, with 7-H_3 signals at 1.80 and 1.79 ppm, respectively, were assigned the

(*6R*)-configuration. By comparison, the downfield 7-H_3 resonances of the (*6S*)-epimers **2.20** and **2.19** occurred at 1.93 and 1.95 ppm, respectively.

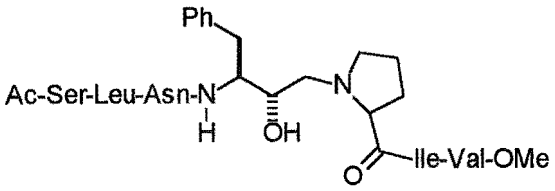
The assignment of the C3 configurations of compounds **3.57/3.58**, **3.61-3.64** and **2.19**, **2.20**, **3.71, 3.72** was made by comparison of the chemical shifts of the 2-H, 3-H, 6-H and 7-H_3 resonances, of the entire series of tetrazole derivatives (table 4.2). Typically, compounds with a (*3S*)-configuration gave 2-H and 3-H resonances in a downfield position, relative to their (*3R*)-epimers. Similarly, compounds with a (*3S*)-configuration gave 6-H and 7-H_3 resonances in an upfield position relative to those of their (*3R*)-epimers. The only exception is seen for **3.71**, which had a 7-H_3 resonance 0.01 ppm downfield relative to (*3R*)-**3.72**. These trends were observed for diastereoisomers at C3, regardless of the configuration at C6. On this basis, the C3 configurations of **3.57**, **3.61**, **3.63**, **2.20** and **3.71** were assigned as *S*, while those of the corresponding (3)-epimers **3.58**, **3.62**, **3.64**, **2.19** and **3.72** were assigned as *R* (table 4.2).

The 3-H resonances of **3.61-3.64** (* table 4.2) were part of a multiplet at 4.57 to 4.68 ppm and were unable to be distinguished from each other (these epimers were not separated). Similarly, the 2-H and 6-H resonances of **3.57** and **3.58** were indistinguishable. Consequently, these resonances were unable to be included in the correlations between chemical shift and configuration.

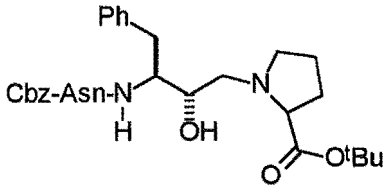
HIV-1 Protease Inhibition

The tetrazole-based inhibitors shown in table 4.3, were tested for *in vitro* HIV-1 protease inhibition. The compounds were tested at the Centre for Drug Design and Development, University of Queensland through the generous support of Dr. David Fairlie. The inhibitors show reasonable activity, with IC_{50} values in the micromolar (μM) range.

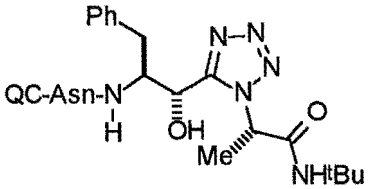
An aim of the preliminary results presented in this thesis was to make some meaningful conclusions about the biological activity of compounds based on our designed *cis*-hydroxyethylamine isostere, such as **2.19**. Some comparisons of HIV protease inhibition can be made between the series of tetrazoles and existing potent inhibitors.



1.19 IC_{50} 6 nM*



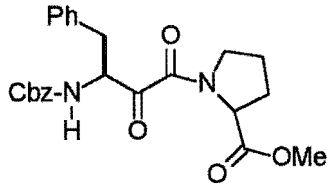
2.22 IC_{50} 0.3 μM



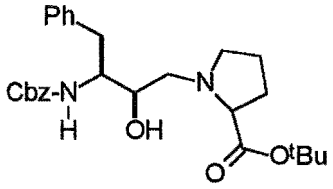
2.19 IC_{50} 60 μM

The most potent known HIV protease inhibitors are significantly more potent, with IC_{50} values in the low nanomolar (nM) range. Compound **1.19**, upon which the design of our HIV protease inhibitors was based, exhibits an IC_{50} of 6 nM (* obtained under the same assay conditions; note that the IC_{50} values of most of the existing inhibitors were obtained under different assay conditions). However, **1.19** has an extended structure spanning from P_4-P_3', giving it greater potency. A more suitable comparison is between the tetrazoles **2.19**, **2.20**, **3.71** and **3.72**, and the

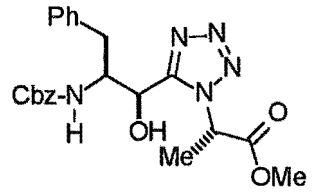
hydroxyethylamine-based inhibitor **2.22**, which all have similar ligands spanning P_3-P_2'. The tetrazoles lack the extended sequence (from P_2') known to favour a *cis*-type geometry at P_1-P_1' (see chapter two, HIV protease inhibition). The tetrazoles **2.19**, **3.71** and **3.72** are approximately 160 fold less active by comparison to **2.22**. It may be that the steric bulk of the tetrazole ring is preventing close proximity in the active site, and therefore hindering stronger binding interactions.[§]



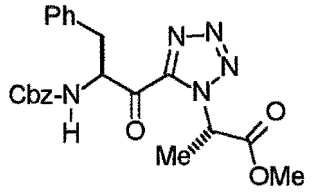
2.16 IC_{50} 0.4 μM



2.25 IC_{50} 6.5 μM



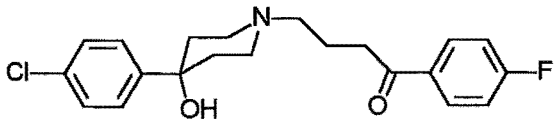
3.65 IC_{50} 460 μM



3.70

A comparison of the potency of our tetrazole core structure with the existing hydroxyethylamine and α-ketoamide core structures, can be made by comparing the activities of **3.65/3.66** with the known inhibitors **2.25** and **2.16**. All of these compounds have the ligand Cbz at P_2 and either O^{t}Bu or OMe at P_2', as *N* and *C* terminals. The tetrazole **3.65** shows reasonable activity by comparison to **2.25**, being 70 fold less potent. However, the α-ketoamide **2.16** is 1100 fold more potent. A more useful comparison to α-ketoamide **2.16** would be the corresponding α-ketotetrazole **3.70**. This compound was synthesized, however its biological activity will be investigated in future studies.

[§] Preliminary modelling studies by Dr. Fairlie at the 3D Centre for Drug Design, University of Queensland suggests a steric problem exists in the active site.



4.1

Our inhibitors do show 60 fold more potent activity than the known inhibitor haloperidol **4.1**, which has an IC_{50} of 3 mM under the same assay conditions. Haloperidol was identified[120] by a structure-based computer search of the Cambridge Crystallographic Database, for compounds which fit in the active site, and is one of the few reported non-peptide based inhibitors of HIV protease.

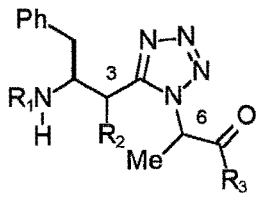


Table 4.3: HIV-1 protease inhibition activity of the tetrazole derivatives.

	R_1	R_2	R_3	C3	C6	IC_{50} (μM)	Ki (μM)
3.49/3.50	Cbz-Asn	H	NH^{t}Bu		*SR*	700 (±200)	200 (±100)
3.53	QC-Asn	H	NH^{t}Bu		*S*	170 (±20)	50 (±20)
3.54	QC-Asn	H	NH^{t}Bu		*R*	300 (±100)	80 (±60)
3.65	Cbz	OH	OMe	*S*	*S*	460 (±80)	140 (±60)
3.66	Cbz	OH	OMe	*R*	*S*	720 (±60)	210 (±70)
2.20	QC-Asn	OH	NH^{t}Bu	*S*	*S*	200 (±80)	60 (±40)
2.19	QC-Asn	OH	NH^{t}Bu	*R*	*S*	60 (±10)	17 (±7)
3.71	QC-Asn	OH	NH^{t}Bu	*S*	*R*	43 (±9)	13 (±6)
3.72	QC-Asn	OH	NH^{t}Bu	*R*	*R*	51 (±3)	15 (±4)

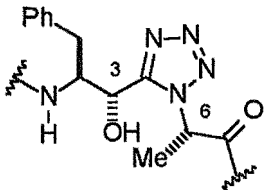
An analysis of the activity data also leads to some conclusions about structure-activity relationships within our series of inhibitors. A comparison of the activities of **3.49/3.50**, **3.53** and **3.54** shows that a clear preference exists for the 2-quinolinylcarbonyl (QC) ligand at P_3 instead of Cbz, as seen previously in comparing the inhibitors **2.14** and **2.23**, based on **1.19** (see table 2.1). The two fold difference in activity between **3.53** and **3.54** suggests that a (*S*)-configuration at C6 is preferred, as

predicted by the modelling studies on our tetrazole system (see chapter two figures 2.9-2.11).

Compounds **3.65** and **3.66**, with only Cbz at P_2, show less activity than **2.19**, **2.20**, **3.71** and **3.72**, which have the favoured QC-Asn ligand at P_3-P_2 for greater recognition. A small difference in activity is also observed between **3.65** and **3.66**, dependent on the configuration of the C3 center. However, it appears that the (*3S*)-configuration of **3.65** is slightly favoured, contrary to the predictions of the modelling studies. It may be that extension of the central tetrazole isostere with suitable ligands at P_2'-P_4' is required to force the targeted bioactive conformation with a (*R*)-configuration at C3, discussed in chapter two.

The most potent of the inhibitors tested were compounds **2.19**, **2.20**, **3.71** and **3.72**, containing both the C3-hydroxyl transition state mimic and more favourable ligands at P_3-P_2 and P_2' (see chapter two). Compounds **2.19**, **3.71** and **3.72** are significantly more active than the corresponding compounds, **3.53** and **3.54**, which lack a C3-hydroxyl group. This is consistent with the C3-hydroxyl group forming crucial hydrogen bonding interactions with the aspartate residues in the active site, as predicted, and acting as a mimic of the transition state of amide bond hydrolysis. A three fold difference in activity is also observed between **2.20** and **2.19**, which both have (*S*)-configurations at C6, but differ in their C3 configurations. It appears here that the (*3R*)-configuration of **2.19** is preferred. However, compounds **3.71** and **3.72**, despite having different configurations at C3 and the previously established, unfavoured (*6R*)-configuration, display equal activity to each other and also to **2.19**. It may be that extension of the inhibitor to P_3' and P_4' is required, to force the binding mode of **1.19** (see discussion in chapter two), before any clear preferences are observed for the (*R*)-configuration at C3. Another factor to consider is the possible steric effects of the bulky tetrazole ring in the active site, which has been suggested above as a cause of the generally less potent activity of the tetrazole series. If the tetrazole ring is preventing close contact in the active site, the binding interactions to the adjacent C3-hydroxyl group may be weakened, causing less significant differences in activity between tetrazoles with different C3 configurations.

Conclusion



cis-hydroxyethylamine isostere **2.12**

A series of HIV protease inhibitors were synthesized, based on the *cis*-hydroxyethylamine peptidomimetic **2.12**. The compounds showed reasonable *in vitro* activity, with IC_{50} values in the micromolar range. Some comparisons were able to be made with existing potent inhibitors of HIV protease. Our designed dipeptide isostere **2.12** showed less potent activity against HIV-1 protease than the α-ketoamide and hydroxyethylamine core structures. It was proposed that the steric bulk of the tetrazole ring was not projecting into the crucial S_1' binding pocket of the active site effectively and was preventing close proximity.

Comparison of the activity within the series of tetrazoles allowed some structure-activity correlations to be made. QC-Asn at P_3-P_2 is better than Cbz-Asn and also Cbz at P_2, as predicted. A (*6S*)-configuration is preferred over *6R* for compounds **3.53** and **3.54**, and between compounds **2.19** and **2.20** a (*3R*)-configuration is preferred over *3S*, as expected. However, compounds **3.65**, **3.66**, **3.71** and **3.72** show no clear trends. It was proposed that extension of structure **2.12** to P_3'-P_4' is required, in order to favour the targeted bend-type binding conformation of known inhibitor **1.19**. Future studies may include lengthening the backbone of **2.12** between the tetrazole ring and the crucial C3-hydroxyl group, to enable the tetrazole ring to bind in S_1' and the hydroxyl group to also still interact with the catalytic aspartate residues. A larger more suitable P_1' ligand, instead of the methyl group of the existing series, may also help to fill the large S_1' pocket of HIV protease. Extension of **2.12** to include P_3' and P_4' ligands is proposed to significantly increase the potency of the structure.

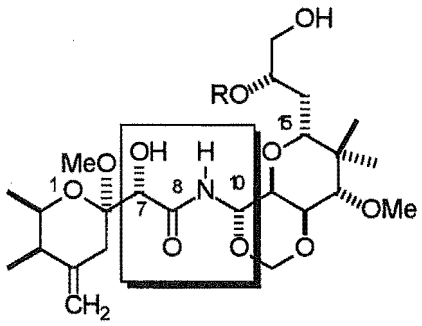
A generally applicable peptidomimetic structure **2.12** has been designed and applied to the inhibition of HIV protease. A general synthesis for **2.12** and its extension in the *N* and *C* directions was developed and a series of compounds with reasonable activity were synthesized.

Chapter Five

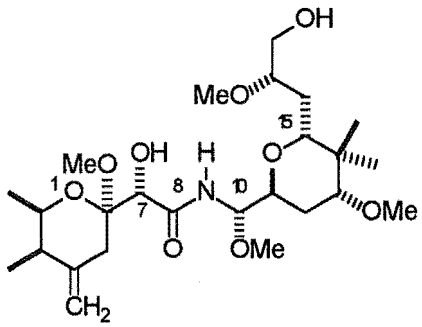
Synthesis of the Mycalamide Analogues

Introduction

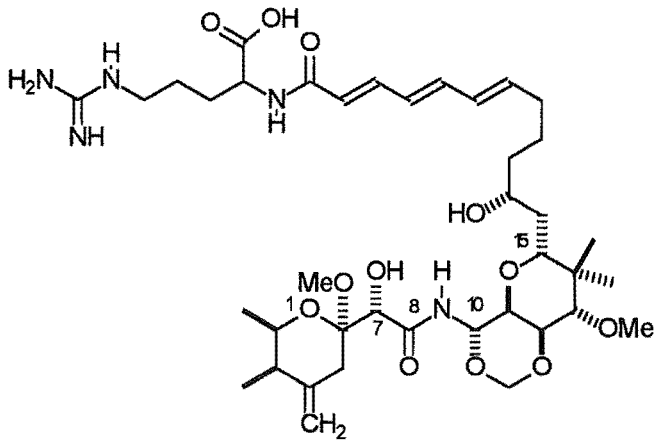
The natural products mycalamides A **5.1**[121] and B **5.2**[122] were discovered, as part of a screening project of New Zealand marine invertebrates, as metabolites from a sponge of the genus *Mycale*, found in Otago Harbour. Bioactivity-directed purification of a large scale, organically soluble extract (409g from 12.8kg of sponge) gave **5.1** (65 mg, IC_{50} 3.0 ng/mL) and **5.2** (20 mg, IC_{50} 0.7 ng/mL), with potent *in vivo* antiviral and antitumour activity.



5.1 R=H
5.2 R=Me



5.3



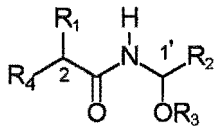
5.4

Compounds **5.1** and **5.2** belong to a class of natural product, including pederin **5.3**,[123] the onnamides[124] (A, **5.4**) and theopederins,[125] which is characterized by the presence of the O1-C10 pederic acid subunit (see structures **5.1-5.4** for numbering) and differs principally in the C15 sidechain. The onnamides and theopederins are also isolated from marine sponges while pederin, a potent insect toxin, is isolated from a beetle (*Paederus fuscipes* sp.). Mechanism of action studies[126] on this class of compound indicate that their ability to inhibit protein synthesis is correlated to their antitumour activity. The bioactivity results of a series of mycalamide derivatives, prepared in this laboratory, also showed that a strong correlation existed between their *in vitro* antiviral and P388 anti-leukaemia activity,[127] supporting the idea of protein synthesis inhibition as a common mechanism of action. It is therefore proposed that **5.1-5.4**, and derivatives, are able to act as peptidomimetics.

An extensive series of microscale structure-activity studies[128a-c] on **5.1** and **5.2** was undertaken with a view to understanding the requirements for biological activity. More than 110 derivatives were tested. Some of the more important structure to activity correlations from this study are summarized in the following. Acylation or alkylation of the 7-OH group caused 10-10^2 fold drops in activity as compared to **5.1**. All derivatives containing an oxazolidinone ring between 7-OH and C10 were at least 10^2 fold less active. Methylation of both the amide nitrogen and 7-OH resulted in a 10^3 fold less active derivative. Cleavage of the C8-N9 amide bond resulted in total loss of activity. The product of deoxygenation at C10 was 40 times less active than **5.1**, suggesting the crucial importance of the C10 configuration to the activity.

Kocienski[129] has also reported that the C10 epimer of mycalamide B (**5.2**) is some three orders of magnitude less active than the parent compound. Further support for the critical importance of the C10 oxygen came from studying the biological activity of the various onnamide and theopederin derivatives that have been isolated by the Fusetani group from *Theonella* sp. sponges.[130a-b] Most notable was the reported inactivity of an onnamide derivative lacking oxygenation at C10.[130a] By comparison, the removal of C18-OH by oxidative decarbonylation resulted in a slightly more active derivative. These experiments demonstrated that the α-hydroxyamidoacetal C7-C10 functionality of **5.1** and **5.2** is essential to their *in vitro* P388 anti-leukaemia activity.

The aim of the research undertaken in this thesis was to synthesize and test , *in vitro* against the P388 leukaemia cell line, simple analogues of the C7-C10 function of the parent compounds **5.1-5.4**. On this basis, compounds of the general structure **5.5** were targeted, where R_1 to R_4 could be variously alkyl, aryl or H and with defined stereochemistry at each of the two stereogenic centers.



5.5

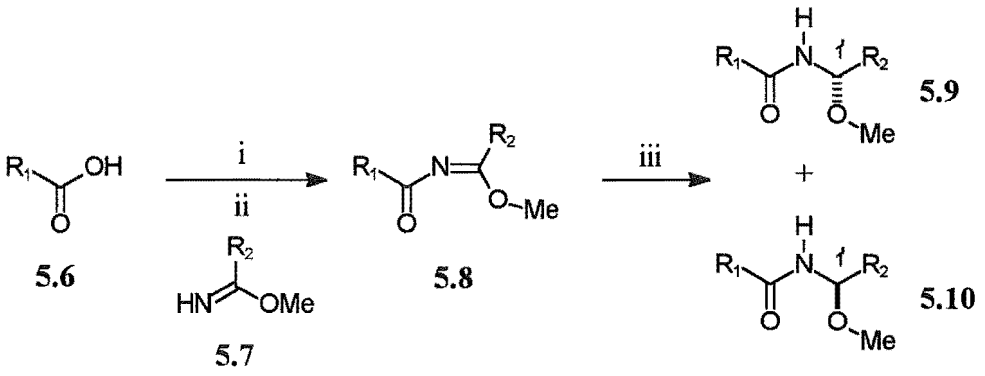
Synthesis of the Mycalamide Analogues

Due to the natural scarcity, challenging structure and potent bioactivity of this class of natural products **5.1-5.4**, significant efforts have been directed towards their total synthesis. Total syntheses of pederin,[131a, 131b] and more recently the mycalamides and onnamide A[132] have been reported.

We have developed two general synthetic routes to analogues based on structure **5.5**. The selection of R_1 to R_4 was based initially on synthetic utility, then on aspects pertaining to the actual structure of the mycalamides, and finally on aspects such as solubility.

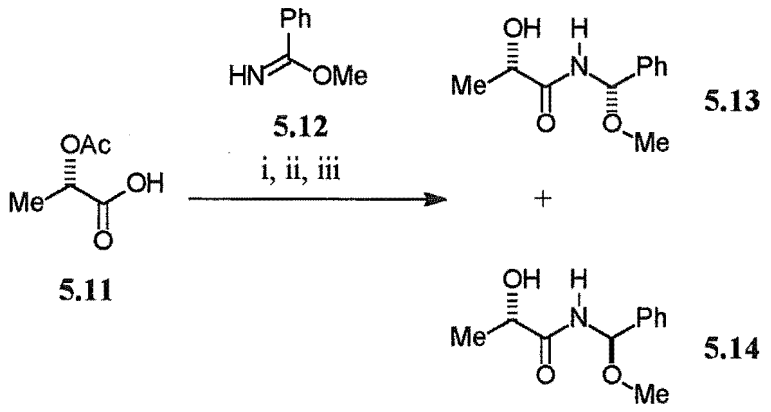
General Method A

General method A (scheme 5.3) was based on procedures developed during the first total synthesis of pederin. Matsumoto[133] reported the first total synthesis of pederin **5.3** via a coupling of the two halves of the molecule to form the C8-N9 amide bond. The procedure involved the coupling of an acid **5.6** with a methyl imidate **5.7** to give the methyl *N*-acylimidate **5.8**. This was reduced with sodium borohydride to give a mixture of the target compound **5.9** and its (1′)-epimer **5.10** (scheme 5.1). During the development of this procedure a number of model systems [based on acid **5.6** = (*2S*)-mandelic acid or (*2RS*)-lactic acid] were investigated, which were pertinent to our target structure **5.5**, having simple R_1-R_4 groups.



Scheme 5.1: **i**, $SOCl_2$, pyridine, CH_2Cl_2, rt; **ii**, **5.7**, Et_3N; **iii**, $NaBH_4$, EtOH or MeOH, 0 °C.

Previously in our laboratories, (*S*)-lactic acid acetate **5.11** was reacted with methyl benzimidate **5.12** according to this procedure to give an epimeric mixture of **5.13** and **5.14** (scheme 5.2). However, the mixture was inactive against P388 murine leukaemia cells, and so compounds based on the readily available (*S*)-3-phenyllactic acid **5.15**, with a different R_4 group of the substructure **5.5**, were targeted. This choice of R_4 was adopted throughout the series of mycalamide analogues (figure 5.1).



Scheme 5.2: **i**, $SOCl_2$, pyridine, CH_2Cl_2, rt, 20 min; **ii**, **5.12**, Et_3N, rt, 80 min; **iii**, $NaBH_4$, EtOH, 0 °C, 4 h.

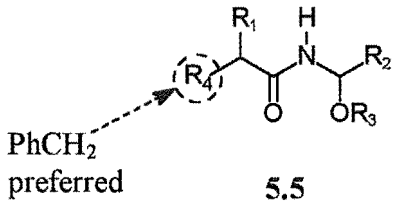
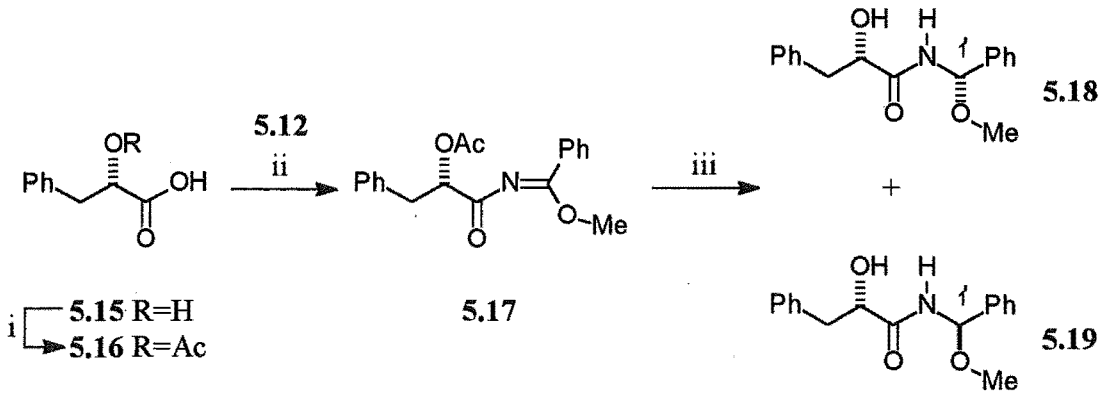


Figure 5.1: Choice of R_4.

The procedure of Matsuda[133], and the similar methodology of Kocienski,[131b] was modified to include a DCC/HOBT mediated coupling of an acid **5.6** and a methyl imidate **5.7**, to give the intermediate **5.8**. Initially, (*S*)-3-phenyllactic acid acetate **5.16** was coupled with methyl benzimidate **5.12** using DCC/HOBT, to give the methyl *N*-acylimidate **5.17**. Reaction of **5.17**, with a large excess of sodium borohydride in isopropyl alcohol (IPA), gave a (1′)-epimeric mixture of **5.18** and **5.19** (1:1 by ^1H NMR) in 33% yield (scheme 5.3). The epimers were separated by silica-based radial chromatography. Perhaps surprisingly, reduction of **5.17** also resulted in removal of the acetate group. Acetate **5.16** was prepared by reaction of acid **5.15** with acetic anhydride in pyridine. Compound **5.12** was prepared by reaction of benzonitrile with methanol and dry hydrogen chloride gas.[134]



Scheme 5.3: Preparation of **5.18/5.19** by general method A; **i**, Ac_2O, pyridine, rt, 3 h; **ii**, **5.12**, DCC, HOBT, Et_3N, CH_2Cl_2, rt, 18 h; **iii**, $NaBH_4$, IPA, rt, 18 h.

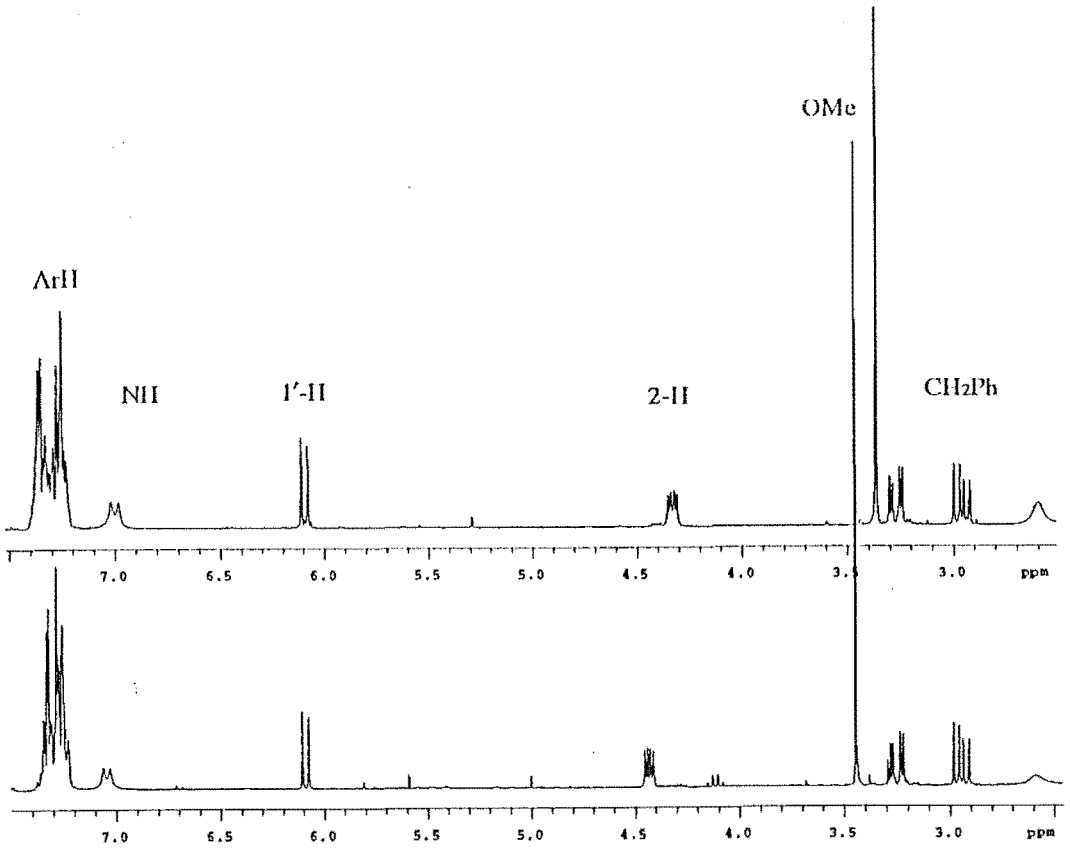
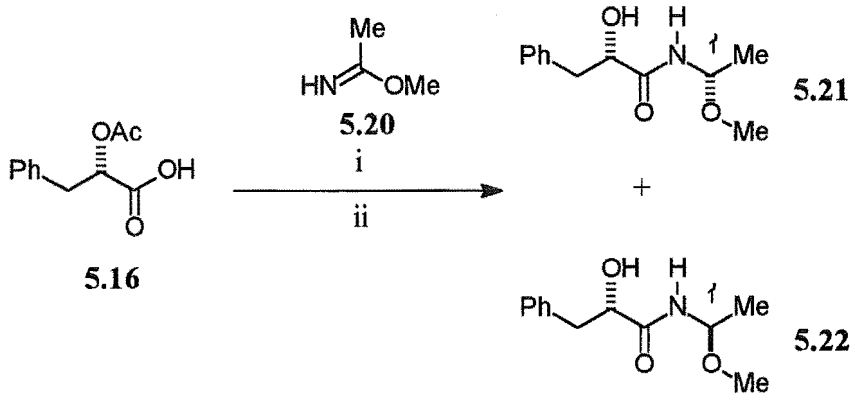


Figure 5.2: ^1H NMR spectra (solvent $CDCl_3$) of **5.18** (top) and **5.19** (bottom).

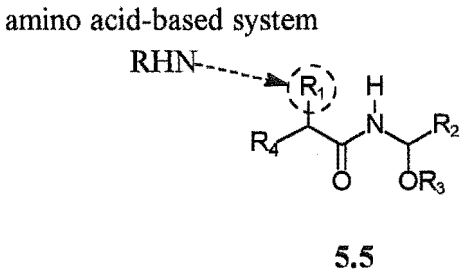
Compounds **5.18** and **5.19** were identified by NMR. Their ^1H NMR spectra, shown in figure 5.1, are characteristic of the entire series of mycalamide analogues that were subsequently prepared. The distinctive methoxyl resonances at 3.37 and 3.47

ppm, and 2-H resonances at 6.10 and 6.12 ppm indicated successful coupling and reduction.

A similar procedure, using commercially available methyl acetimidate **5.20**, gave a (1′)-epimeric mixture of **5.21** and **5.22** (1:1 by ^{1}H NMR) in 42% yield (scheme 5.4). The epimers were partially separated by silica-based radial chromatography. This reaction gave analogues with a alternative R_2 group, based on structure **5.5**.



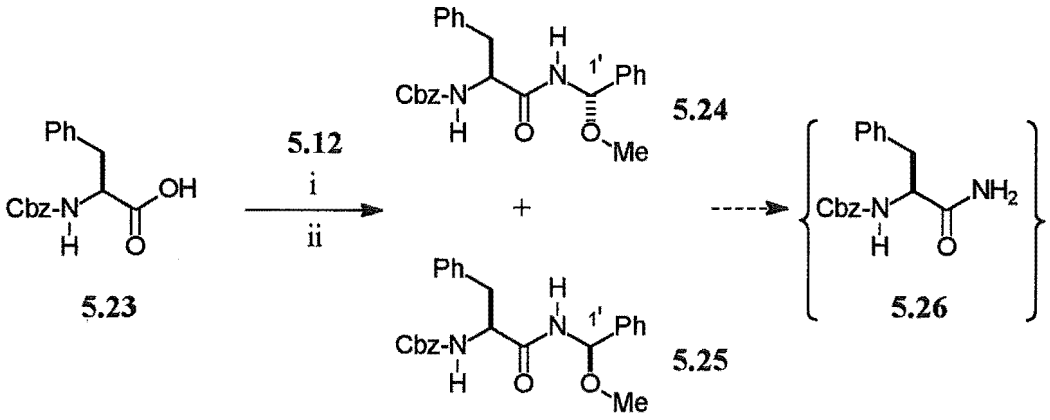
Scheme 5.4: **i**, **5.20**, DCC, HOBT, Et_3N, CH_2Cl_2, rt 18 h; **ii**, $NaBH_4$, IPA, rt, 18 h.



5.5

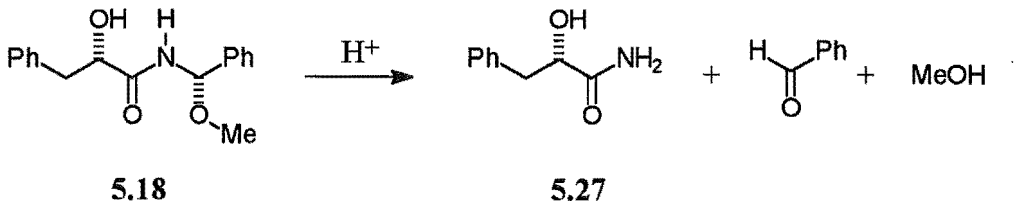
Figure 5.3: Extension to amino acid based systems.

After the development of general method A using the acid **5.15**-based system, an investigation of its application to amino acid-based systems was carried out (figure 5.3). A similar procedure, using Cbz-*L*-Phe **5.23** and imidate **5.12**, gave a (1′)-epimeric mixture of **5.24** and **5.25** (1:1 by ^{1}H NMR) in 42% yield (scheme 5.5). The epimers were separated by silica-based radial chromatography.



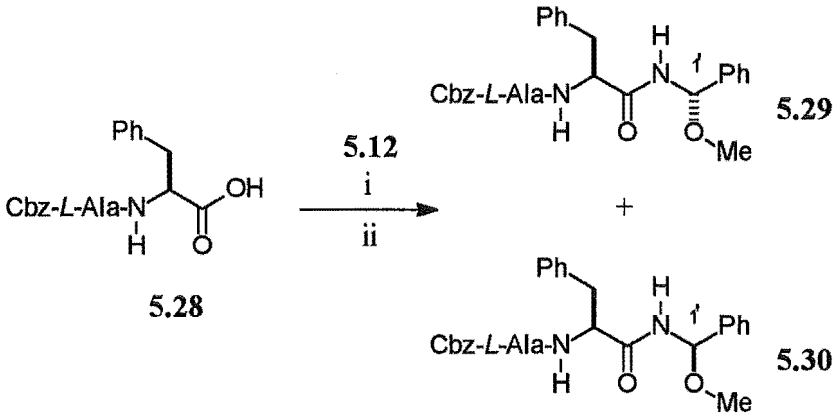
Scheme 5.5: **i**, **5.12**, DCC, HOBT, Et_3N, CH_2Cl_2, rt 18 h; **ii**, $NaBH_4$, IPA, rt, 18 h.

N-Cbz-*L*-phenylalaninamide[146] **5.26** was isolated in 37% yield during the purification and separation of **5.24** and **5.25**. Amide **5.26** was proposed to be formed by an acid-catalyzed decomposition of the amidoacetal function on silica. This characteristic decomposition was investigated by monitoring the reaction of **5.18** in slightly acidic $CDCl_3$, by ^1H NMR, over 14 days (scheme 5.6). After 24 hours the appearances of a singlet at 3.49 ppm corresponding to methanol, and a singlet at 10.04 ppm, corresponding to the benzaldehyde proton, were observed. Over 14 days, the signals for **5.18** completely receded and signals corresponding to (*S*)-3-phenyllactamide **5.27** appeared and increased with time. The signals for methanol and benzaldehyde increased over time in conjunction with the appearance of **5.27**. The instability of the amidoacetal function was also observed during the development of the total syntheses of the parent compounds **5.1-5.4**.[135]



Scheme 5.6: Acid-catalyzed decomposition of amidoacetal functionality.

In an extension of the peptide-based system, Cbz-*L*-Ala-*L*-Phe **5.28** and the imidate **5.12** were reacted according to the general method A to give a (1′)-epimeric mixture of **5.29** and **5.30** (1:1 by ^{1}H NMR) in 54% yield (scheme 5.7). The epimers were separated by silica-based radial chromatography.

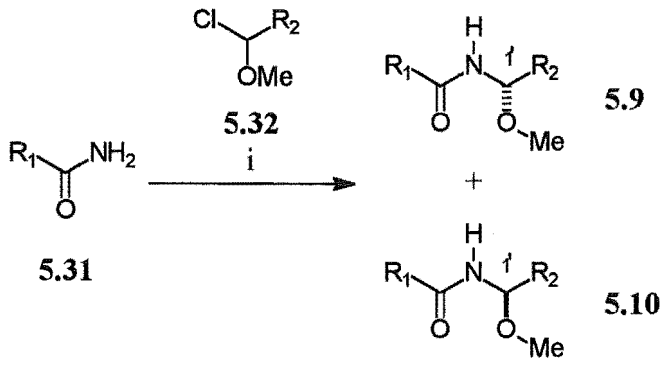


Scheme 5.7: **i**, **5.12**, DCC, HOBT, Et_3N, CH_2Cl_2, rt 18 h; **ii** $NaBH_4$, IPA, rt, 18 h.

General Method B

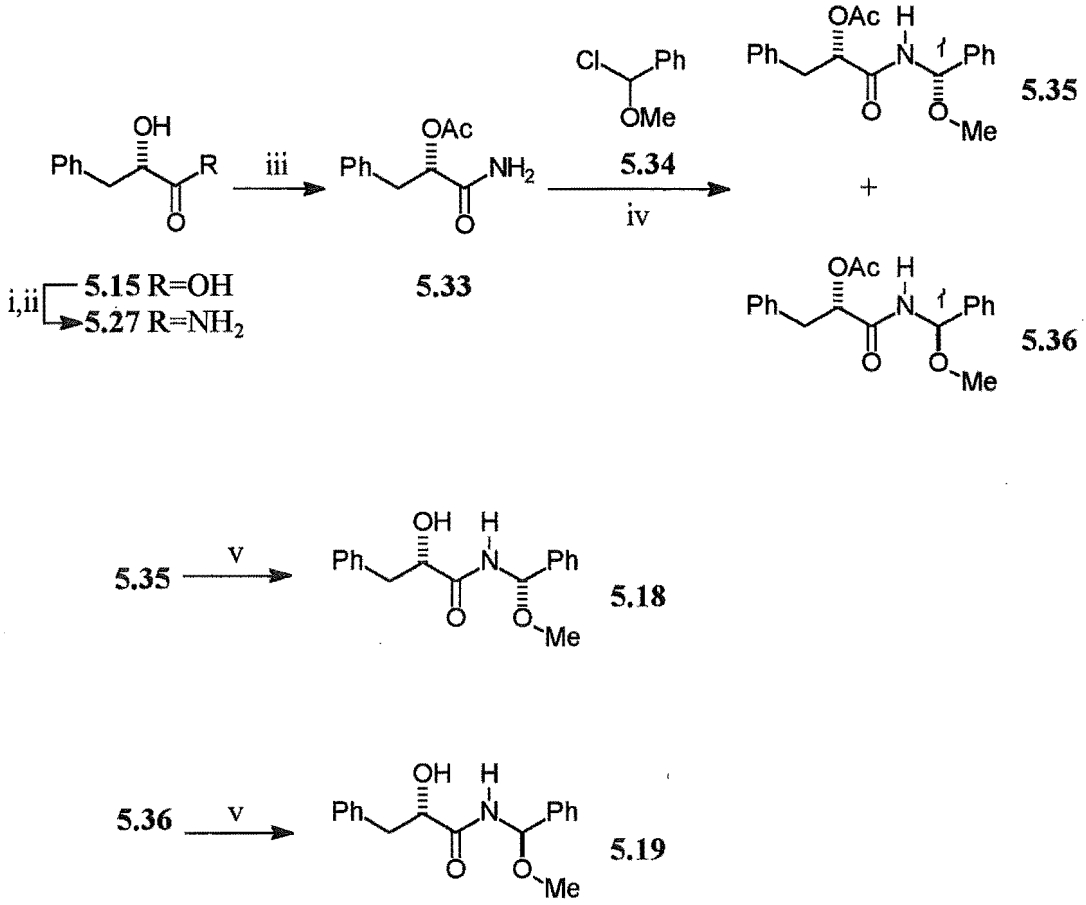
An alternative synthesis, to general method A, of the target structure **5.5**, was investigated in an effort to develop a higher yielding and cleaner coupling reaction. Some useful discussions with Professor Sir John Cornforth at this time, lead to ideas for a new synthetic route.

General method B involved the reaction of a dichloromethane solution of the primary amide **5.31** with an α-chloro ether **5.32**, in the presence of triethylamine, to give directly a (1′)-epimeric mixture of **5.9** and **5.10** (scheme 5.8).



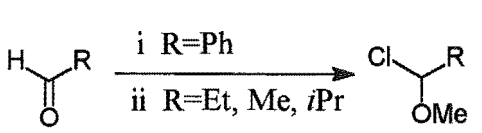
Scheme 5.8: General Method B; **i**, **5.32**, Et_3N, CH_2Cl_2, 0 °C.

In order to compare the two general methods, the primary amide **5.33** was reacted with the α-chloro ether **5.34**, using this procedure, to give a (1′)-epimeric mixture of **5.35** and **5.36** in 83% combined yield (scheme 5.9). The epimers were separated by a combination of silica-based radial chromatography and recrystallization. Hydrolysis of the acetates **5.35** and **5.36** with 0.2 equivalents of potassium carbonate in methanol-water gave the corresponding hydroxyl compounds **5.18** and **5.19** in 95% and 90% yields, respectively.



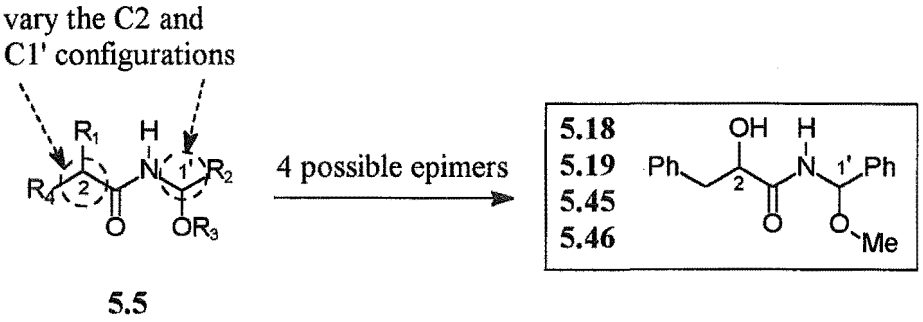
Scheme 5.9: **i**, acetone, H_2SO_4, -10 °C; **ii**, NH_3, rt, 18 h; **iii**, Ac_2O, pyridine, rt, 3 h; **iv**, **5.34**, Et_3N, CH_2Cl_2, 0 °C, 18 h; **v**, K_2CO_3, MeOH, H_2O, rt 2 h.

The acetate **5.33** was prepared by reaction of amide **5.27** with acetic anhydride in pyridine. The amide itself was prepared[136] by condensation of acid **5.15** with acetone followed by reaction with ammonia (scheme 5.9, steps i and ii). A mixture of the racemic α-chloro ether **5.34** and benzaldehyde (17:3 by ^{1}H NMR) was prepared[137] (scheme 5.10), by reaction of benzaldehyde with methanol and dry hydrogen chloride gas in ethyl chloride at -60 °C, and was used in the coupling reaction without further purification (scheme 5.9, step iv). Mixtures of the racemic α-chloro ethers **5.37-5.39** and the corresponding aldehydes (17:3 to 9:1 by ^{1}H NMR) were prepared by a similar procedure involving reaction of the aldehyde with methanol and dry hydrogen chloride gas at -30 °C. These mixtures were used in subsequent coupling reactions without further purification.



5.34 R=Ph
5.37 R=Et
5.38 R=Me
5.39 R=*i*Pr

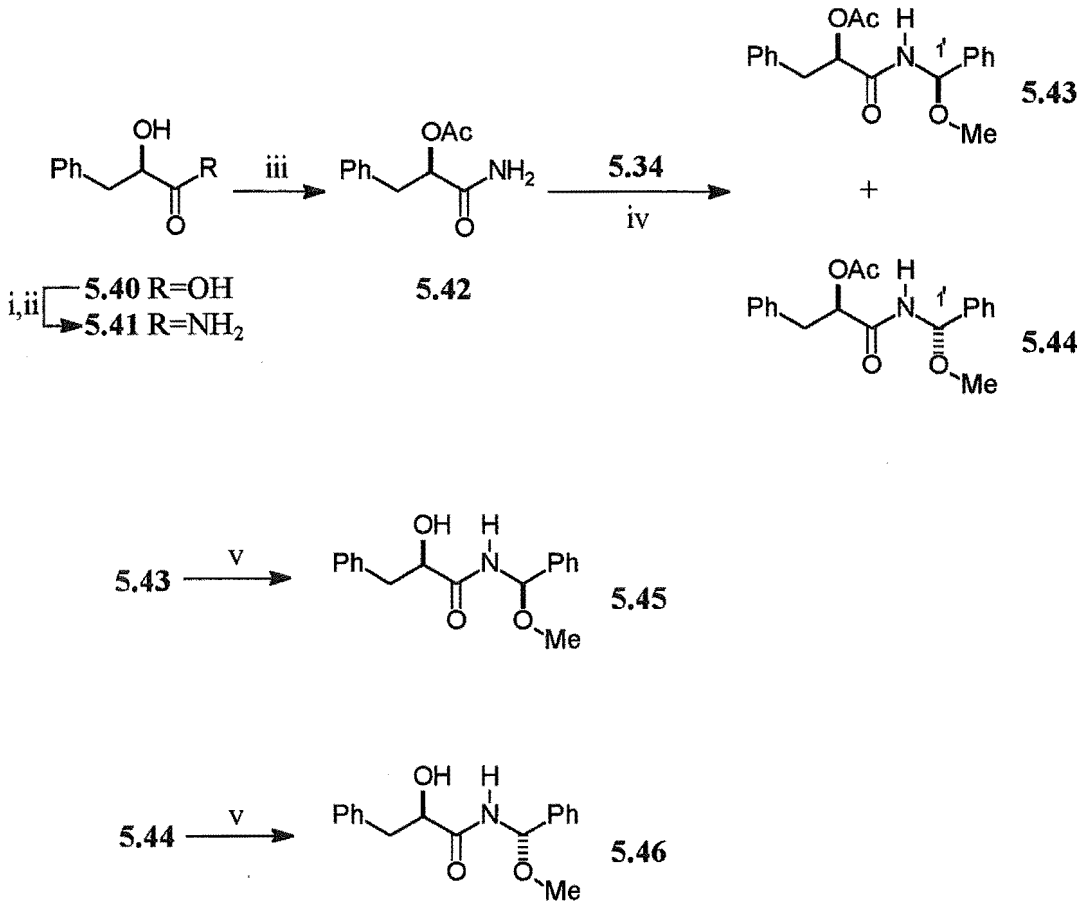
Scheme 5.10: **i**, MeOH, $HCl_{(g)}$, EtCl, -60 °C, 1 h; **ii**, MeOH, $HCl_{(g)}$, -30 °C.



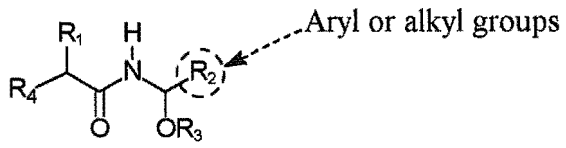
5.5

Figure 5.4: Configurational expansion of the series of analogues.

In order to investigate fully the effects of the C2 and C1′ configurations of the analogues (figure 5.4) on bioactivity, the (*2R*)-epimers of **5.18** and **5.19** were targeted for synthesis. The acetate **5.42** (the enantiomer of **5.33**) was reacted with **5.34** according to general method B to give a (1′)-epimeric mixture of **5.43** and **5.44** (1:1 by ^{1}H NMR) in 41% yield (scheme 5.11). The epimers were separated by a combination of silica-based radial chromatography and recrystallization. Hydrolysis of the acetates **5.43** and **5.44** with potassium carbonate (as for **5.35/5.36** above) gave **5.45** and **5.46** (enantiomers of **5.18** and **5.19**) in 60% and 84% yields, respectively. This meant that all four possible epimers of the system, **5.18/5.19/5.45/5.46**, were able to be tested for bioactivity (for a discussion see chapter six). The acetate **5.42** was prepared from (*R*)-3-phenyllactic acid **5.40** via amide **5.41**, by a similar procedure[136] to the preparation of **5.33** above.



Scheme 5.11: **i**, acetone, H_2SO_4, -10 °C; **ii**, NH_3, rt, 18 h; **iii**, Ac_2O, pyridine, rt, 3 h; **iv**, **5.34**, Et_3N, CH_2Cl_2, 0 °C, 18 h; **v**, K_2CO_3, MeOH, H_2O, rt 2 h.

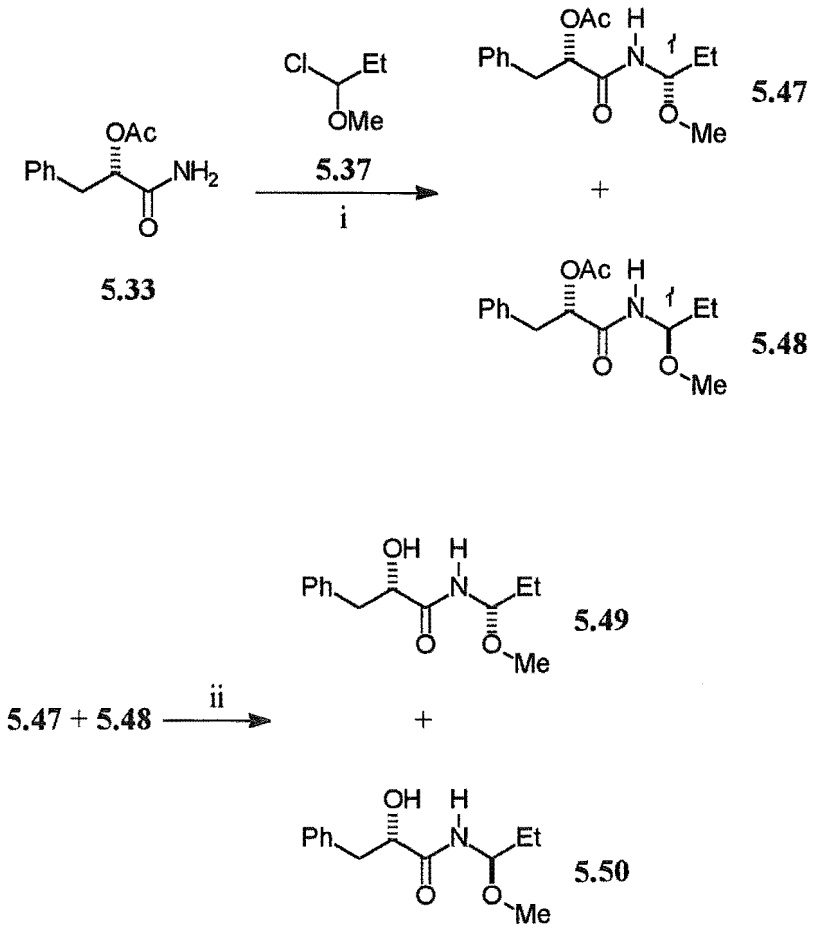


5.5

Figure 5.5: Expansion of the series of analogues by altering R_2.

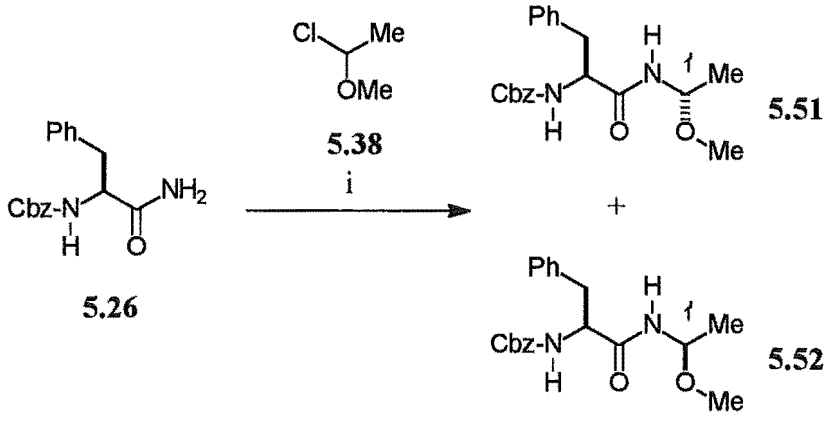
The amide **5.33** and α-chloro ether **5.37** were reacted according to general method B to give a (1')-epimeric mixture of **5.47** and **5.48** in 70% yield, to provide an expansion of the series of analogues at R_2 (figure 5.5),. The mixture was hydrolyzed with 0.2 equivalents of potassium carbonate in methanol-water to give a mixture of the

corresponding hydroxyl compounds **5.49** and **5.50** in 92% yield (scheme 5.12). The epimers were separated by silica-based radial chromatography.



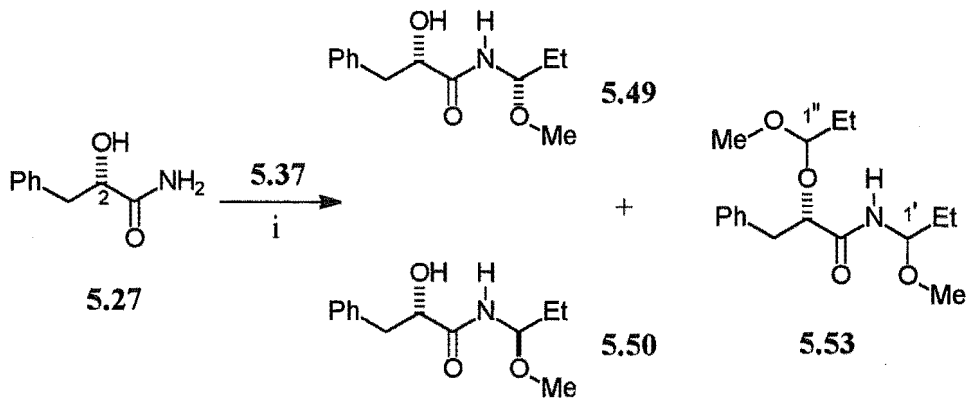
Scheme 5.12: **i**, **5.37**, Et_3N, CH_2Cl_2, 0°C, 18 h; **ii**, K_2CO_3, MeOH, H_2O, rt 2 h.

As in the development of general method A above, an investigation of the application of general method B to amino acid-based systems was carried out. Cbz-*L*-Phe-NH_2 **5.26** and α-chloro ether **5.38** were reacted according to general method B to give a (1′)-epimeric mixture of **5.51** and **5.52** in 52% yield (scheme 5.13). The epimers were separated by silica-based radial chromatography.



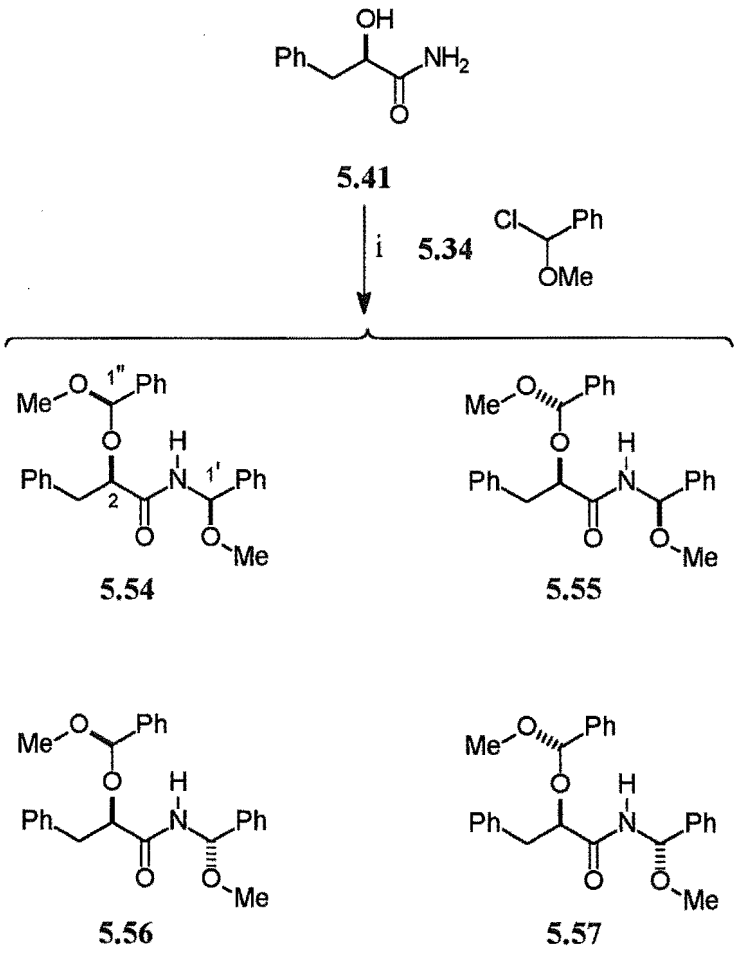
Scheme 5.13: **i**, **5.38**, Et_3N, CH_2Cl_2, 0 °C, 18 h.

During the development of general method B, the reactions of α-hydroxyamides **5.27** and **5.41** directly with α-chloro ethers **5.37** and **5.34**, respectively, in the presence of triethylamine, were investigated. Reaction of a dichloromethane solution of **5.27** with 3 equivalents of **5.37** and triethylamine gave **5.49** and **5.50** in 43% combined yield and a (1′,1″)-epimeric mixture of four compounds, identified by ^{1}H NMR as **5.53** (24% yield) (scheme 5.14). Some amide **5.27** (22%) was reisolated after purification on silica, as a result of acid-catalyzed decomposition of **5.49/5.50** (discussed above). The mixture of products obtained in this reaction suggests that coupling of the α-chloro ether with the primary amide is preferred over the alternative C2-hydroxyl group coupling.



Scheme 5.14: **i**, **5.37**, Et_3N, CH_2Cl_2, 0 °C, 18 h.

The reaction of a dichloromethane solution of amide **5.41** with a larger excess of **5.34** (>15 equiv) and triethylamine gave a (1′,1″)-epimeric mixture of **5.54-5.57** in 79% combined yield (scheme 5.15). These adducts are formed by coupling of both the amide NH_2 and C2-hydroxyl groups with the α-chloro ether, as above. Compounds **5.54-5.57** were identified by characteristic ^1H and ^{13}C NMR signals. By comparison of the ^1H NMR spectra of **5.54-5.57** with derivatives **5.45** and **5.46**, extra resonances were observed at 2.93-3.22 ppm for the (1″)-methoxyl singlet and at 5.02-5.44 ppm for the 1″-H singlet (see figure 5.6). In the ^{13}C NMR spectra, the distinctive resonances of C1″ were observed at 103.21-104.76 ppm. The epimers **5.54-5.57** were partially separated by silica-based radial chromatography. After the observation of these adduct products, coupling by general method B was carried out on hydroxyl-protected amides, to prevent the possibility of coupling at the C2-hydroxyl group.



Scheme 5.15: **i**, **5.34**, Et_3N, CH_2Cl_2, 0 °C, 18 h.

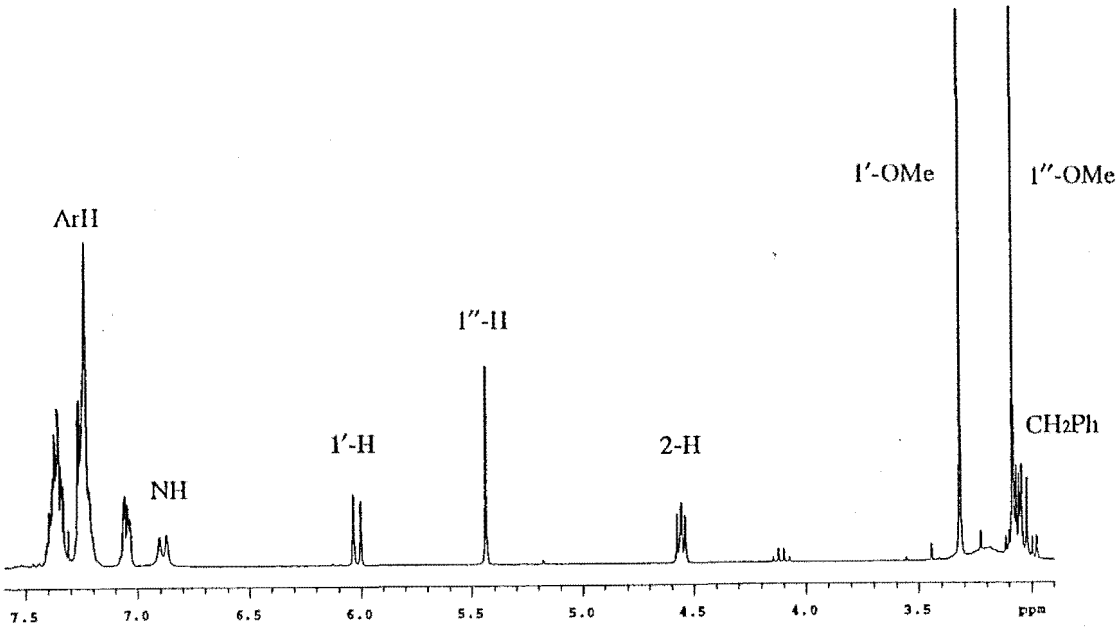
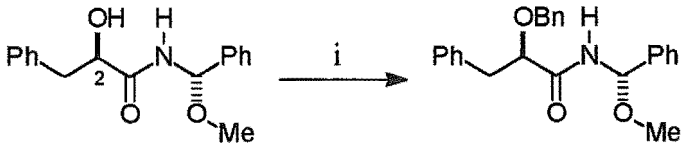


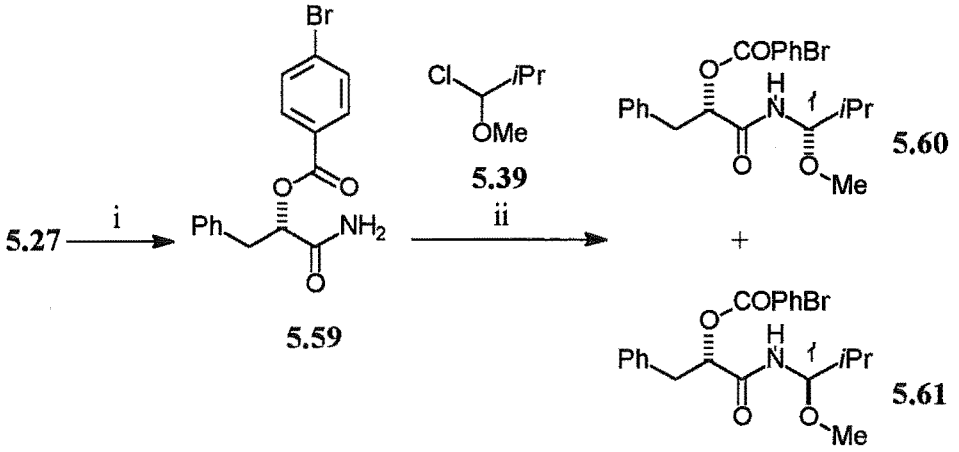
Figure 5.6: ^{1}H NMR spectrum (solvent $CDCl_3$) of one of the epimers **5.54-5.57** (unassigned).



5.46 **5.58**

Scheme 5.16: i, PhCOCl, 4-DMAP, Et_3N, CH_2Cl_2, rt, 2 d.

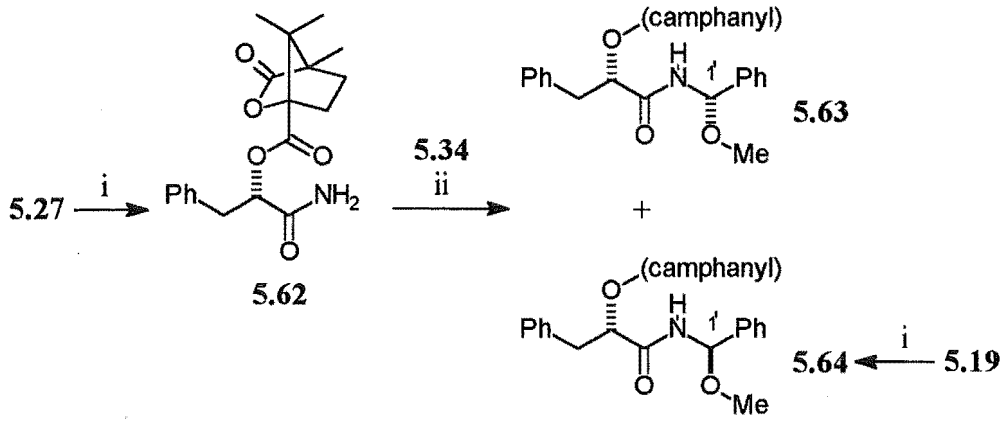
In addition to expanding the series of analogues for structure activity studies, attempts were made to prepare compounds suitable for X-ray crystallographic analysis. On this basis, ester derivatives of the 2-hydroxyl function were prepared. Compound **5.46** was benzoylated by reaction with benzoyl chloride, in the presence of 4-dimethylaminopyridine and triethylamine, to give **5.58** (scheme 5.16). During attempts at recrystallization of the product, some interesting crystals of (4-dimethylaminopyridine)$_5$(benzoic acid)$_3$(H_2O)$_{10}$ were obtained which were suitable for X-ray crystallography, and which sparked further investigation[138] (see chapter seven).



Scheme 5.17: **i**, 4-BrPhCOCl, 4-DMAP, Et_3N, CH_2Cl_2, rt, 3 h; **ii**, **5.39**, Et_3N, CH_2Cl_2, rt, 3 h.

In a further attempt to obtain a suitable crystalline derivative, amide **5.27** was reacted with 4-bromobenzoyl chloride, 4-dimethylaminopyridine and triethylamine to give the ester **5.59**. The ester was reacted with α-chloro ether **5.39** according to general method B to give a crude mixture of **5.60** and **5.61** (1:1 by ^{1}H NMR) (scheme 5.17). The (1′)-epimers were purified and partially separated by silica-based radial chromatography, giving a 12% combined yield (lowered due to some acid-catalyzed decomposition).

In a similar procedure, the amide **5.27** was reacted with (*1S,4R*)-camphanic chloride, 4-dimethylaminopyridine and diisopropylamine in dichloromethane to give the camphanate **5.62**. This was reacted with α-chloro ether **5.34** according to general method B to give a (1′)-epimeric mixture of **5.63** and **5.64** (1:1 by ^{1}H NMR) in 51% yield (scheme 5.18). The epimers were partially separated by silica-based radial chromatography. Compound **5.63** was crystallized from an epimeric mixture to give crystals suitable for X-ray crystallography (see figure 6.7). A mixture of **5.64** and **5.63** (9:1 by ^{1}H NMR) was also prepared from a mixture of **5.19** and **5.18** (9:1 by ^{1}H NMR) using (*1S,4R*)-camphanic chloride as described for the preparation of **5.62**. This conversion provided a correlation between the configurations of **5.18/5.19** and **5.63/5.64**, respectively, from which followed the configurations of the entire series of analogues (for a discussion see chapter six).

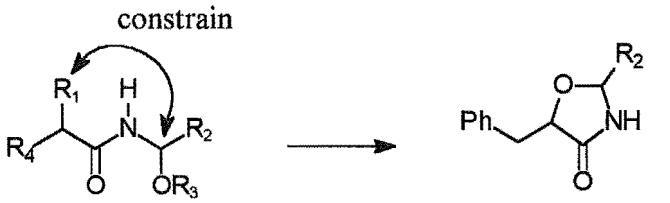


Scheme 5.18: **i**, (*1S,4R*)-camphanic chloride, 4-DMAP, *i*Pr$_2$NEt, CH_2Cl_2, rt, 18 h; **ii**, **5.34**, Et_3N, CH_2Cl_2, -78 °C, 18 h.

A comparison of general methods A and B shows that method B proved superior to method A, for the preparation of derivatives based on **5.5**. In particular, coupling of the amide **5.26**, **5.33**, **5.42** or **5.62** with the α-chloro ether **5.34**, **5.37**, **5.38** or **5.39**, to form analogues of general structure **5.5**, gave typical yields ranging from 83% (for **5.35/5.36**) to 51% (for **5.63/5.64**). The corresponding two step procedure of general method A, coupling **5.16**, **5.23** or **5.28** with **5.12** or **5.20**, proved less satisfactory and gave typical yields ranging from 54% (for **5.29/5.30**) to 33% (for **5.18/5.19**). Both general methods required the separation of mixtures of (1′)-epimeric products by silica-based chromatography, which resulted in a reduction in yield, due to acid-catalyzed degradation of the amidoacetal functionality (discussed above). However, sufficient material was able to be obtained for biological testing, so alternative separation methods were not investigated.

Conformationally Restrained Analogues

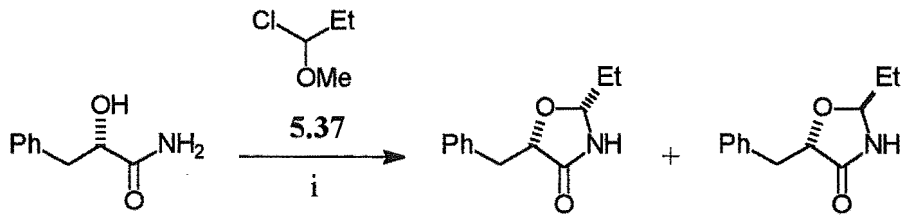
Conformational restriction is an important aspect of peptidomimetics (discussed earlier in chapters one and two), which has been applied in the development of ligands, as probes of the bioactive conformation required for receptor binding. A series of conformationally constrained cyclic oxazolidinones, based on the target structure **5.5**, were prepared on this basis (figure 5.7). In these compounds the amide bond is forced to adopt a *cis* configuration. The configuration of the amide bond is essential to the bioactivity of peptides and peptidomimetics, as discussed in chapter one. A related technique of *cis* conformational restriction is discussed in chapters two through four.



5.5 oxazolidinone

Figure 5.7: Conformational restriction of structure **5.5**.

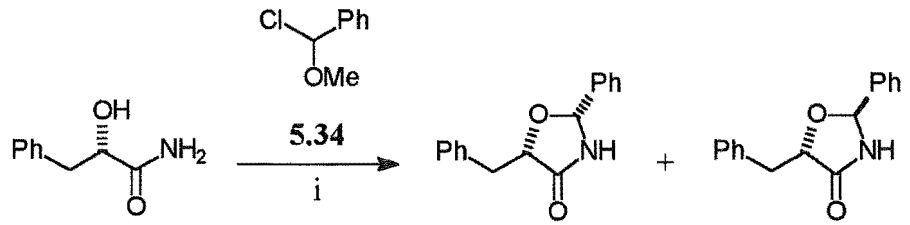
A dichloromethane solution of the amide **5.27** was reacted with the α-chloro ether **5.37** in the absence of triethylamine to give a mixture of *trans*-**5.66** and *cis*-**5.65** oxazolidinones (13:7 by ^{1}H NMR) (scheme 5.19). Separation of the isomers by silica-based radial chromatography gave a 17% combined yield of **5.65** and **5.66**. A literature[139] synthesis of 2,5-substituted-oxazolidin-4-ones involves the condensation of α-hydroxyamides or cyanohydrins with carbonyl compounds in the presence of an acid catalyst.



5.27 **5.65** **5.66**

Scheme 5.19: **i, 5.37**, CH_2Cl_2, rt, 18 h.

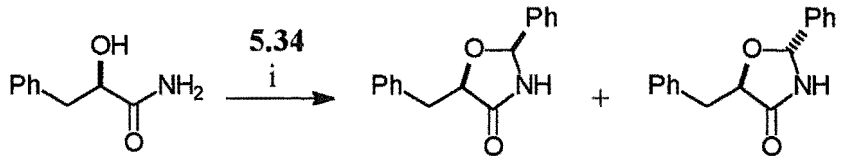
A similar procedure using **5.34** gave a mixture of *trans*-**5.68** and *cis*-**5.67** (2:1 by ^1H NMR) (scheme 5.20). Separation of the isomers by silica-based radial chromatography gave a 53% combined yield of **5.67** and **5.68**.



5.27 **5.67** **5.68**

Scheme 5.20: **i, 5.34**, CH_2Cl_2, rt, 18 h.

The enantiomers of **5.68** and **5.67** were prepared by a similar procedure using the amide **5.41** and the α-chloro ether **5.34** to give a mixture of *trans*-**5.70** and *cis*-**5.69** (3:2 by ^1H NMR) (scheme 5.21). Separation of the isomers by silica-based radial chromatography gave a 46% combined yield.



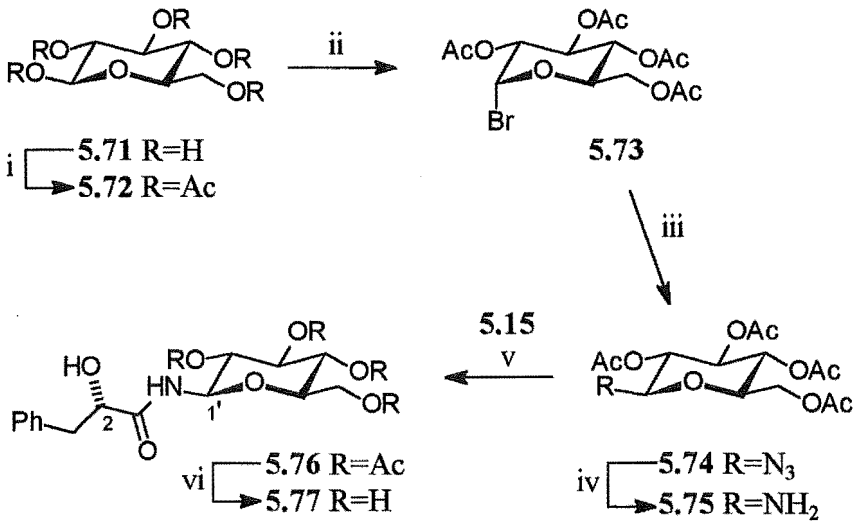
5.41 **5.69** **5.70**

Scheme 5.21: **i, 5.34**, CH_2Cl_2, rt, 18 h.

Glucosyl-based Analogues

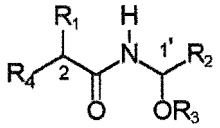
The glucosyl derivatives **5.76** and **5.77** (scheme 5.22) were synthesized as compounds possessing the previously established, biologically active (*1'R*)-configuration (see chapter six for a discussion of bioactivity). As well as modelling more closely the structural requirements of the mycalamide skeleton cf. **5.1**, it was also considered that the sugar moiety might impart improved water solubility, which would be of assistance in the *in vitro* cytotoxicity assay. A glucose-derived amino acid (Gum) has also been developed as a peptidomimetic and incorporated into cyclic somatostatin analogues, with potent activity.[140]

Analogue **5.76** was prepared (scheme 5.22) by the coupling of acid **5.15** with glucosylamine **5.75** using DCC/HOBT (59% yield). Hydrolysis of the acetates with potassium carbonate gave the hydroxyl derivative **5.77** in 96% yield. Removal of the acetate groups did improve the water solubility of the analogue, as expected. The precursor 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosylamine **5.75** was prepared from *D*-glucose **5.71** in four steps. Reaction of **5.71** with acetic anhydride and concentrated sulphuric acid gave the pentaacetate **5.72** which was reacted immediately with hydrogen bromide in acetic acid to give the glucopyranosyl bromide **5.73** in 94% yield.[141] This was converted to the azide **5.74** (72% yield) by reaction with sodium azide in formamide.[142] Catalytic hydrogenation of the azide gave the required amine **5.75** in 85% yield.[143]



Scheme 5.22: **i**, Ac_2O, H_2SO_4, 100 °C, 2 h; **ii**, HBr-AcOH, 0 °C to rt, 3 h; **iii**, NaN_3, $HCONH_2$, 80 °C, 3 h; **iv**, H_2, PtO_2, EtOAc, rt, 3.5 h; **v**, **5.15**, DCC, HOBT, CH_2Cl_2, rt, 5 d; **vi**, K_2CO_3, MeOH, H_2O, rt, 30 min.

Conclusion



5.5

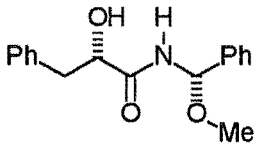
Two general methods were developed in the synthesis of a series of analogues of general structure **5.5**. These methods gave (1′)-epimeric mixtures of analogues, which were separated by rapid silica-based chromatography, despite undergoing characteristic acid-catalyzed decomposition. Sufficient material was obtained for testing of *in vitro* activity against P388 murine leukaemia cells. A discussion of the biological activity and assignment of configuration of the epimeric analogues follows in chapter six.

Chapter Six

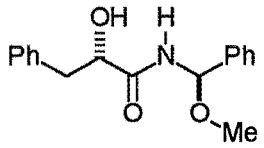
Biological Activity and Assignment of Configuration of the Mycalamide Analogues

Molecular Modelling

Molecular modelling studies were carried out on the reference compounds **5.18** and **5.19** in the Macromodel modelling program, with a view to assigning the unknown C1′ configurations of the series of analogues based on **5.5** (see chapter five). Structures **5.18** and **5.19** were energy minimized in the Amber forcefield with $CHCl_3$ solvation. Monte Carlo conformational searching, with a 3 kcal/mol energy window and 8 variable torsion angles (shown in figure 6.1), gave a series of conformers for both structures **5.18** and **5.19**.



5.18



5.19

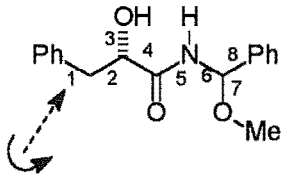


Figure 6.1: Variable torsion angles (1-8) during the conformational search on structures **5.18** and **5.19**.

Further refinements of the modelled conformers were made, such as a more accurate energy minimization of each structure to a lower gradient, and a mathematical test to exclude those structures not in a local minima of the potential energy surface. As a result, a Boltzman distribution of modelled conformers of **5.18** and **5.19** was calculated, based on the relative energies of the conformers within each series. The lowest energy conformers of modelled **5.18** and **5.19** are shown in figures 6.2 and 6.3, respectively.

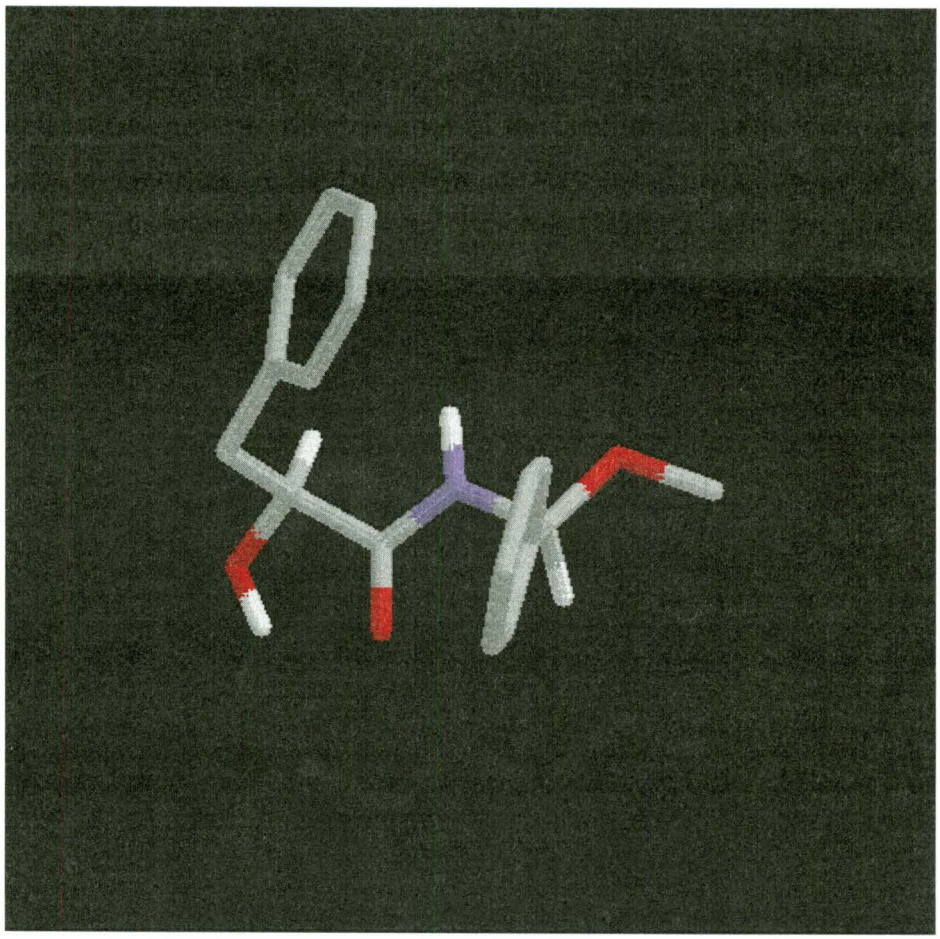


Figure 6.2: Lowest calculated energy conformer of modelled**5.18**.

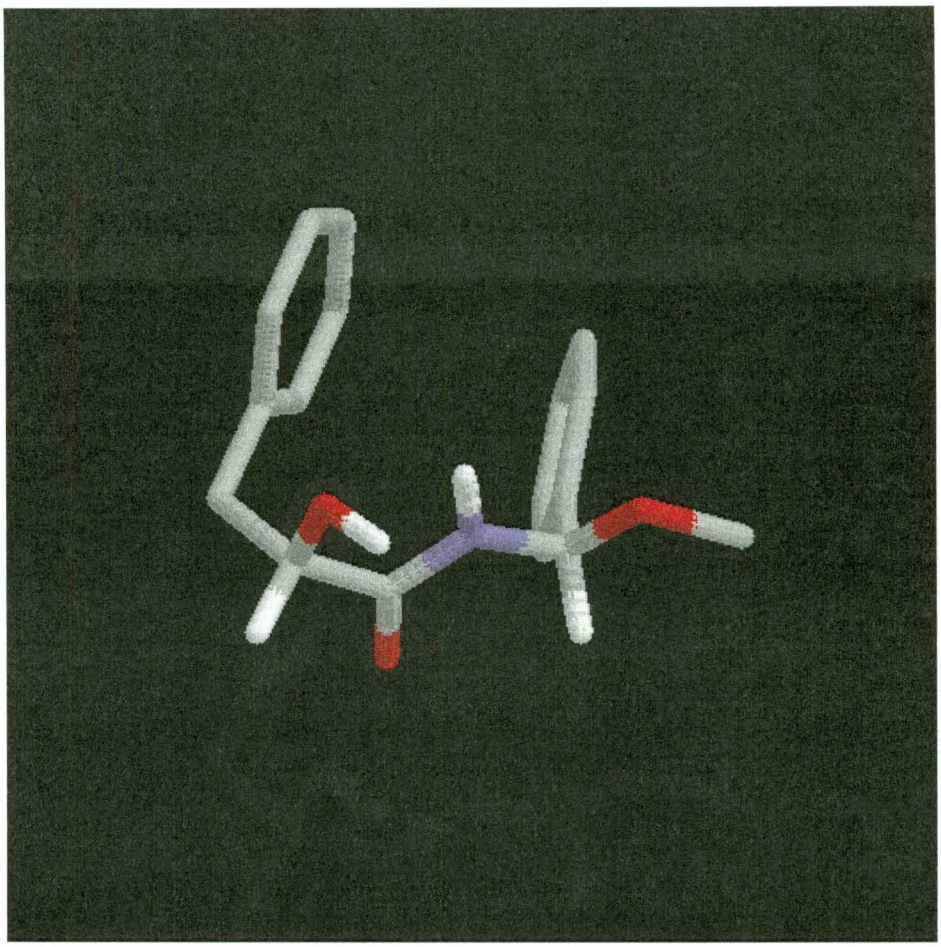
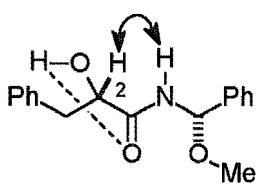
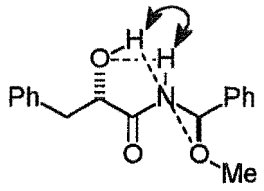


Figure 6.3: Lowest calculated energy conformer of modelled**5.19**.

It was hoped that some correlations could be made between the observed NMR data and the modelled structures, which would enable an assignment of the (1′)-configurations of **5.18** and **5.19**. Some differences in conformation do exist between the modelled epimeric structures, particularly in the torsion angles centered on the C2-C1 and C2-OH bonds (see torsion angles 4 and 3, figure 6.1). Intramolecular distances between relevant protons in the modelled structures of **5.18** and **5.19** are shown in table 6.1 (the distances given are characteristic of the most significant conformers). The molecular modelling studies suggest that some differences in observable nOe effects may exist between the two epimers. Most significantly, it was proposed that a positive nOe existed between 2-H and NH in **5.18** which would not be observed in **5.19**, and that one existed between OH and NH in **5.19** which would not be observed in **5.18** (figure 6.4).



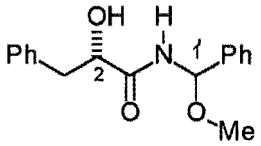
5.18



5.19

Figure 6.4: Proposed intramolecular hydrogen bonding (dashed lines) and nOe (solid arrows) interactions in the modelled structures **5.18** and **5.19**.

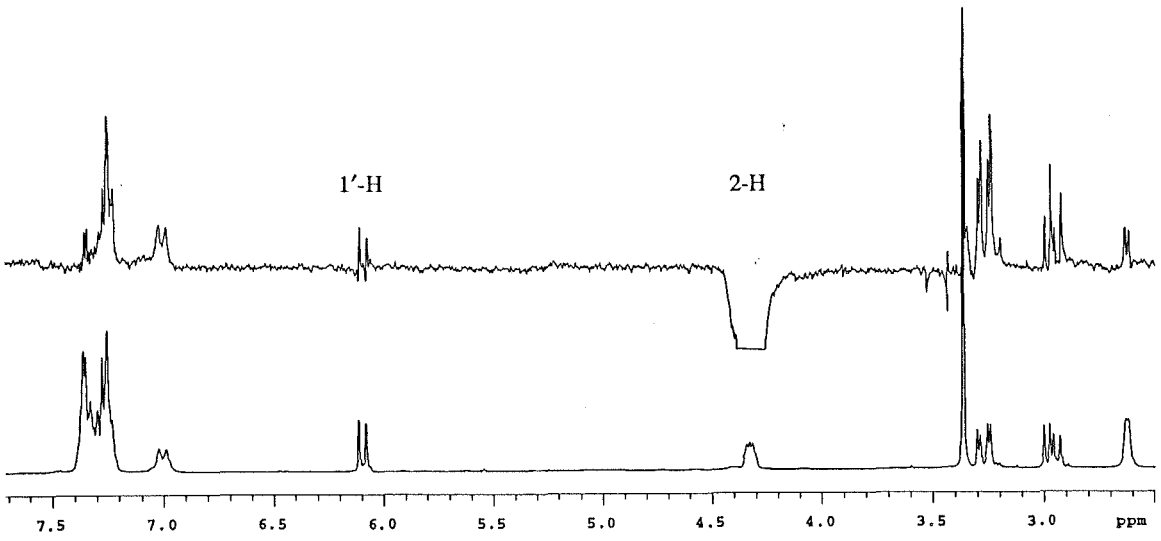
However, comparison to the NMR data of **5.18** and **5.19** showed no significant correlations. Positive nOe effects were observed for both **5.18** and **5.19** between 2-H and NH, and also between OH and NH. One difference between the NMR data of **5.18** and **5.19** that was observed was a small positive nOe between 2-H and 1′-H for **5.18**, which was not observed for **5.19** (figure 6.5). However, this observation could not be correlated to the modelled structures, where the intramolecular distance between 2-H and 1′-H, in both **5.18** and **5.19**, is comparable (table 6.1).



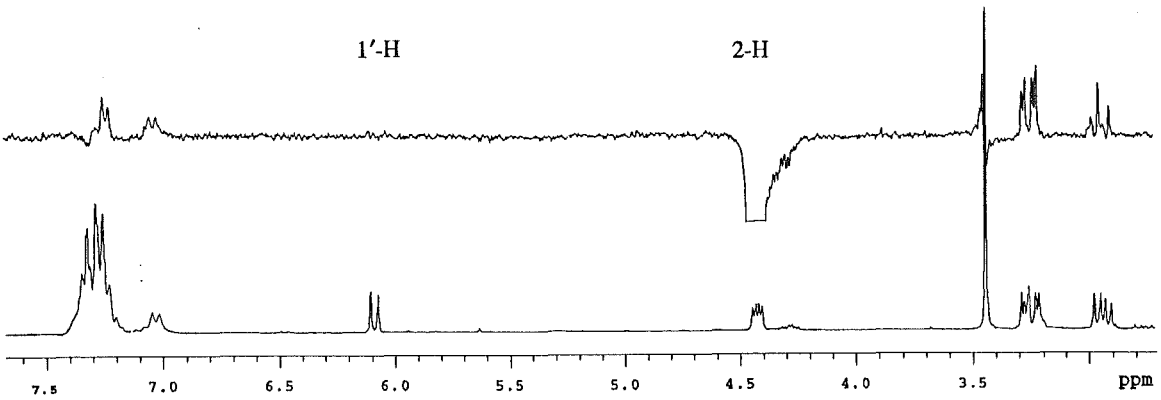
(*1′S*)-**5.18**
(*1′R*)-**5.19**

Table 6.1: Molecular modelling studies of **5.18** and **5.19**.

	Intramolecular distances in modelled structures (Å)		Observed nOe effects in NMR data	
	5.18	**5.19**	**5.18**	**5.19**
2-H to N**H**	2.5	3.4	+	+
OH to N**H**	4.1	2.1	+	+
2-H to 1′-H	4.4	4.6	+	–
OH to **CO**	1.9	3.6		
OH to N**H**	3.9	2.2		



Epimer **5.18**



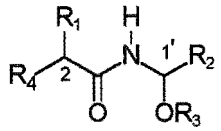
Epimer **5.19**

Figure 6.5: Observable positive nOe for **5.18** between 2-H and 1'-H, not seen for **5.19**.

Different intramolecular hydrogen bonding arrangements are seen in the modelled structures of **5.18** and **5.19** (figure 6.4 and table 6.1). Structure **5.18** has a hydrogen bond between the **O**H and carbonyl **C**O, while **5.19** has hydrogen bonding between **O**H and N**H**, and **O**H and **O**Me (weaker at 4.0 angstroms). The effect of hydrogen bonding on the absorption bands of different functional groups can be observed by infrared spectroscopy. The position of the carbonyl stretching absorption

band is determined by a number of factors, including intermolecular and intramolecular hydrogen bonding, and electronic and mass effects of neighboring substituents. Likewise, the distinctive OH stretching and NH bending absorption bands might be expected to be shifted, relatively, if differences in intramolecular hydrogen bonding exist between epimers. However, compounds **5.18** and **5.19** gave identical infrared data for the OH stretching (3406 cm^{-1}), CO stretching (1684 cm^{-1}) and NH bending (1504 cm^{-1}) bands.

Assignment of Configuration



5.5

The series of analogues based on structure **5.5** were assigned absolute configurations at C2 and C1′, by correlations (figure 6.6) to the absolute configuration of camphanate derivative **5.63**. The relative configuration of **5.63** was determined unambiguously by single crystal X-ray analysis (figure 6.7). The absolute configuration of **5.63**, and hence its (1′)-epimer **5.64**, followed from the known absolute configurations of **5.27** and (*1S,4R*)-camphanyl chloride, which were used to prepare **5.63** and **5.64** (scheme 5.18). The absolute configuration of **5.19**, and hence its (1′)-epimer **5.18**, was assigned on the basis that **5.19** was converted into **5.64**. Compounds **5.45** and **5.46** gave identical NMR data, but opposite optical rotations, to their enantiomers **5.18** and **5.19**. The acetates **5.35**, **5.36**, **5.43** and **5.44** were assigned on the basis of their direct conversion, by hydrolysis, to the reference compounds **5.18**, **5.19**, **5.45** and **5.46**.

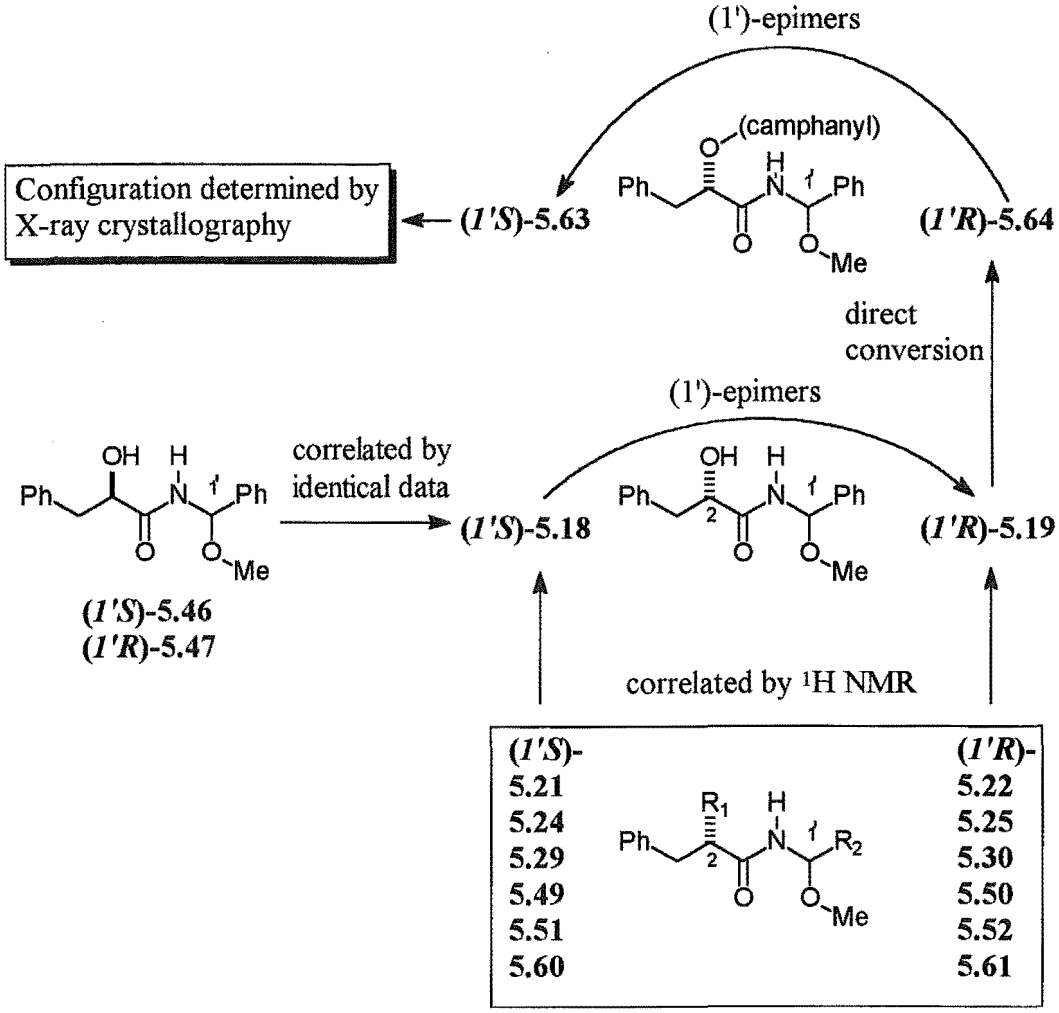


Figure 6.6: Schematic representation of the correlations of the configurations of the series of analogues to the absolute configuration of **5.63**.

The configurations of the other analogues, given in table 6.2, followed by a comparison of their ^{1}H NMR data with that of the reference compounds **5.18** and **5.19**. The methoxyl resonance of the (*1'R,2S*)-derivatives **5.19**, **5.22**, **5.25**, **5.50**, **5.52** and **5.61** was in a characteristic downfield position (0.06 to 0.12 ppm) relative to the corresponding (*1'S,2S*)-epimers **5.18**, **5.21**, **5.24**, **5.49**, **5.51** and **5.60**. The 2-H resonance of the (*1'R,2S*)-derivatives **5.19**, **5.22, 5.50** and **5.61**, where R_1 = OH or OCOPhBr, and R_2 = Ph, Et or Me, was also consistently 0.05-0.13 ppm downfield relative to **5.18**, **5.21**, **5.49** and **5.60**. A further trend was observed in the relative polarity of the epimers on silica. (*1'R,2S*)-Derivatives **5.19**, **5.22, 5.50** and **5.61** consistently eluted after their (1')-epimers **5.18**, **5.21**, **5.49** and **5.60**.

In the amino acid based compounds **5.25**, **5.30** and **5.52**, where R_1 = NHCbz and R_2 = Ph or Me, an opposite trend to above was observed with the 2-H resonance occurring 0.07 to 0.08 ppm upfield relative to their (*1'S*)-epimers **5.24**, **5.29** and **5.51**. The methoxyl resonances of epimers **5.29** and **5.30** were too similar to include in the correlations.

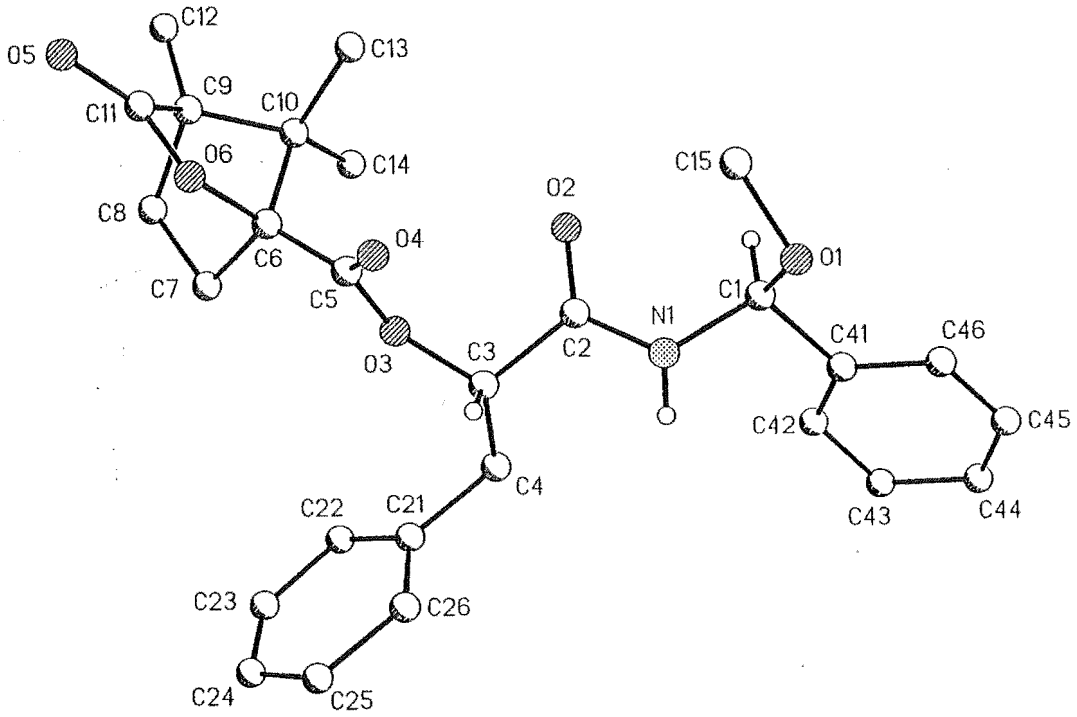


Figure 6.7: X-ray crystal structure of **3.63**.

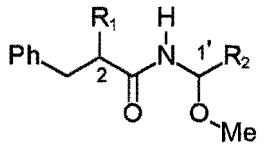
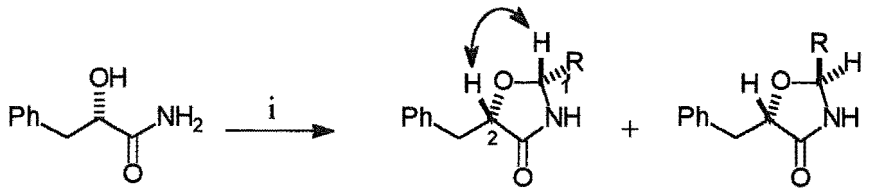


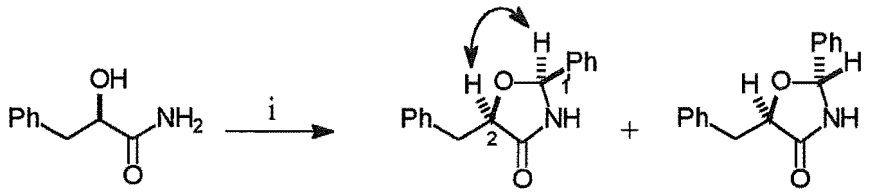
Table 6.2: Assignment of configuration based on ^{1}H NMR spectroscopic data (solvent $CDCl_3$).

Compound	R_1	R_2	1′	2	δ OMe	δ 2-H	Elution
5.18	OH	Ph	*S*	*S*	3.37	4.32	1
5.19	OH	Ph	*R*	*S*	3.47	4.45	2
5.21	OH	Me	*S*	*S*	3.24	4.36	1
5.22	OH	Me	*R*	*S*	3.30	4.41	2
5.24	NH-Cbz	Ph	*S*	*S*	3.32	4.60	1
5.25	NH-Cbz	Ph	*R*	*S*	3.40	4.53	2
5.29	NH-Ala-Cbz	Ph	*S*	*S*	3.31	4.83	2
5.30	NH-Ala-Cbz	Ph	*R*	*S*	3.30	4.76	1
5.45	OH	Ph	*R*	*R*	3.37	4.32	1
5.46	OH	Ph	*S*	*R*	3.47	4.45	2
5.49	OH	Et	*S*	*S*	3.22	4.38	1
5.50	OH	Et	*R*	*S*	3.31	4.46	2
5.51	NH-Cbz	Me	*S*	*S*	3.12	4.55	2
5.52	NH-Cbz	Me	*R*	*S*	3.24	4.47	1
5.60	OCOPhBr	*i*Pr	*S*	*S*	3.16	5.60	1
5.61	OCOPhBr	*i*Pr	*R*	*S*	3.22	5.64	2

The relative configurations of the cyclic oxazolidinone derivatives were assigned on the basis of an observed positive nOe between the ring protons labelled 1′-H and 2-H (non-systematic numbering) of *cis*-isomers **5.65**, **5.67** and **5.69**, but not *trans*-isomers **5.66**, **5.68** and **5.70**. The absolute configurations followed from the known configurations of the amides **5.27** and **5.41**, used to prepare these derivatives (scheme 6.1). Compounds **5.69** and **5.70** gave identical NMR data to their enantiomers **5.67** and **5.68**.



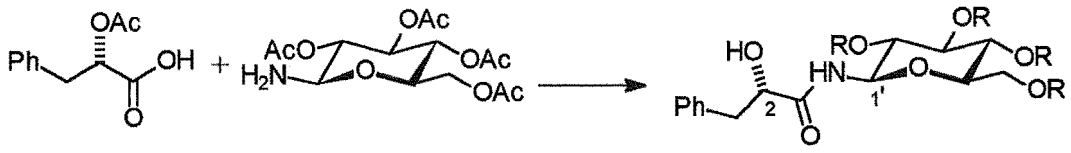
5.27 **5.65** R=Et **5.66** R=Et
5.67 R=Ph **5.68** R=Ph



5.41 **5.69** **5.70**

Scheme 6.1: Observed nOes and assignment of configurations of **5.65-5.70**.

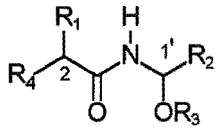
The configurations of the glucosyl analogues **5.76** and **5.77** followed from the known absolute configurations of the acid **5.16** and amine **5.75** used to prepare these compounds.



5.16 **5.75** **5.76** R=Ac
5.77 R=H

Biological Activity

The analogues shown in table 6.3 were each tested for *in vitro* cytotoxicity against P388, a murine leukaemia cell line. IC_{50} values for each sample were determined after incubation for 72 hours with P388 cells. The concentration of sample required to reduce the P388 cell growth by 50% (comparative to control cells) was determined using the absorbance values obtained when the yellow dye MTT tetrazolium is reduced by healthy cells to the purple colour of MTT formazan.[144]



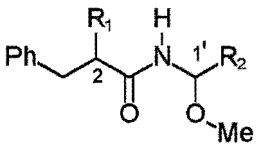
5.5

All of the analogues exhibited far less activity (IC_{50} 8-400 μg/mL) when compared to the parent natural products **5.1** and **5.2** (IC_{50} 3.0 and 0.7 ng/mL, respectively), however the results were still encouraging. In general, compounds based on structure **5.5** with a (*1'R,2S*)-configuration (**5.19**, **5.25**, **5.30** and **5.36**) showed significantly greater *in vitro* cytotoxicity than the corresponding (*1'S,2S*)-derivatives (**5.18**, **5.24**, **5.29** and **5.35**), such that a (*1'R*)-configuration would appear to be favourable towards activity (table 6.3). A notable exception was **5.49** and **5.50** (see below for discussion). An analysis of the data for **5.45** and **5.46**, which possess the alternative (*2R*)-configuration, also suggests that the (*1'R*)-configuration of **5.45** is favoured over the (*1'S*)-configuration of **5.46** for cytotoxic activity. The (*1'R*)-compounds **5.19** and **5.46** show similar *in vitro* antitumour activity such that there seems that there is no marked preference for either a (*R*)- or (*S*)-configuration at C2. The natural products **5.1-5.4**, which are the basis for this model system, possess a (*S*)-configuration at the equivalent C7 centre. It should also be noted that **5.19** and its enantiomer **5.46** have similar activity.

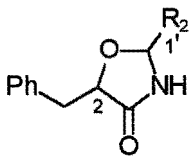
A preference for a (*1'R*)-configuration over a (*1'S*)-configuration (non-systematic numbering) does not seem to be evident within the cyclic oxazolidinone

series **5.65-5.70**, based on the analysis that the *cis* compounds **5.65**, **5.67** and **5.69** all possess similar activity to their *trans* isomers **5.66**, **5.68** and **5.70**. The (*2R*)-compounds **5.69** and **5.70** show greater *in vitro* antitumour activity than their (*2S*)-enantiomers **5.65** and **5.66**, suggesting a preference for a (*2R*)-configuration. In general, the conformationally restrained cyclic analogues **5.65-5.70** showed comparable or better activity to the acyclic derivatives. If these analogues are all interacting with the same receptor(s), then the cyclic derivatives could be explored as useful probes of favourable ligand conformations and functions.

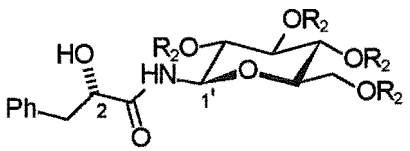
A variety of R_1 groups appear to be accommodated for the induction of *in vitro* cytotoxicity. For example, the corresponding acetates of **5.18** and **5.19**, compounds **5.35** and **5.36**, show comparable activity. The camphanyl and 4-bromobenzoyl ester derivatives **5.64/5.63** and **5.61/5.60** also show comparable activity to reference compound **5.19**. However, the (*1'R*)-derivatives **5.64** and **5.61** do not show significantly greater *in vitro* antitumour activity over their (*1'S*)-epimers **5.63** and **5.60**, as discussed above.



Compounds numbered between **17** and **63**



5.65-5.70



5.76/5.77

Figure 6.7: General structures of the mycalamide analogues shown in table 6.3.

Table 6.3: IC_{50} values of mycalamide analogues against P388 cells.

Compound	R_1	R_2	1′	2	IC_{50} (μg/cm^3)
5.18	OH	Ph	*S*	*S*	>340
5.19	OH	Ph	*R*	*S*	52
5.21	OH	Me	*S*	*S*	>188[a]
5.22	OH	Me	*R*	*S*	>125
5.24	NH-Cbz	Ph	*S*	*S*	>188[b]
5.25	NH-Cbz	Ph	*R*	*S*	14
5.29	NH-Ala-Cbz	Ph	*S*	*S*	>125
5.30	NH-Ala-Cbz	Ph	*R*	*S*	36
5.35	OAc	Ph	*S*	*S*	>313
5.36	OAc	Ph	*R*	*S*	101
5.45	OH	Ph	*R*	*R*	27
5.46	OH	Ph	*S*	*R*	102
5.49	OH	Et	*S*	*S*	43
5.50	OH	Et	*R*	*S*	176
5.51	NH-Cbz	Me	*S*	*S*	>375
5.52	NH-Cbz	Me	*R*	*S*	>375
5.60	OCOPhBr	*i*Pr	*S*	*S*	105
5.61	OCOPhBr	*i*Pr	*R*	*S*	105[c]
5.63	Ocamphanyl	Ph	*S*	*S*	78[d]
5.64	Ocamphanyl	Ph	*R*	*S*	42[d]
5.65		Et	*R*	*S*	57
5.66		Et	*S*	*S*	47
5.67		Ph	*R*	*S*	57
5.68		Ph	*S*	*S*	37
5.69		Ph	*S*	*R*	12
5.70		Ph	*R*	*R*	8
5.76		Ac	*R*	*S*	267
5.77		H	*R*	*S*	>375

a activity obtained on a 1:1 mixture of epimers

b activity obtained on a 3:1 mixture of epimers

c activity obtained on a 17:3 mixture of epimers

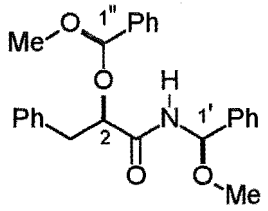
d activity obtained on a 9:1 mixture of epimers

The amino acid based (1′)-epimeric pairs **5.24/5.25**, **5.29/5.30** and **5.51/5.52** (R_1 = NHR), were designed to give analogues with more peptidic character. This was done since the natural products **5.1-5.4**, upon which the series of analogues was modelled, exert their biological activity by an inhibition of protein synthesis. Therefore analogues which act as peptidomimetics may be able to exploit the activity of the parent compounds. The most bioactive compounds in this series (R_1 = NHR) **5.25** and **5.30** show activities comparable to, or better than **5.19**, where R_1 = OH. The introduction of an amino group at R_1, instead of an alcohol group, also enables extension of the analogue as a peptide chain. No significant loss of activity was observed in going from the dipeptide analogue **5.25** to the tripeptide analogue **5.30**. Again a clear preference for the (*1′R*)-configuration of **5.25** and **5.30** is observed over the (*1′S*)-configuration of epimers **5.24** and **5.29**, in an analysis of the activity data.

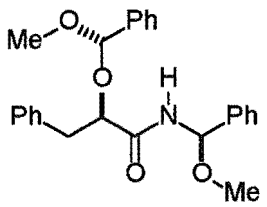
A change from R_2 = Ph to Et appears to be tolerated for compounds **5.19** and **5.49**, although in this case, contrary to other compounds given in table 6.3, a (*1′S*)-configuration seems to give the most potent *in vitro* bioactivity (compare epimers **5.49** and **5.50**). It should be noted that the C2 and C1′ centers of **5.49** possess the same relative configurations of the corresponding C7 and C10 centers of the parent natural products **5.1-5.4**. The oxazolidinone derivatives **5.65** and **5.66**, where R_2 = Et, also show comparable activities to the analogues **5.67** and **5.68**, where R_2 = Ph.

The glucosyl derivatives **5.76** and **5.77** show less activity than the corresponding R_2 = Ph and Et analogues. It was hoped that the sugar moiety would closely resemble the C10-C12 ring of the parent natural products **5.1** and **5.2**, and that this would lead to relatively favourable activity. Furthermore, that the improved water solubility of the sugar might be of assistance in the *in vitro* cytotoxicity assay. However, hydrolysis of the tetraacetate **5.76** to give **5.77**, which did have improved water solubility, resulted in a less active derivative. This may reflect a need to penetrate a hydrophobic cell membrane in the *in vitro* assay.

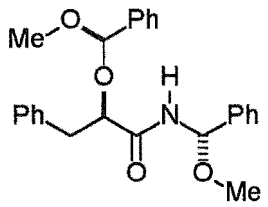
The four diastereoisomers **5.54-5.57** where R_1 = OCH(OMe)Ph all show comparable or better activity (table 6.4) to **5.19** where R_1 = OH (table 6.3). In an analysis of the activity of these compounds there appears to be no preference for the configuration of any one of the four diastereoisomers. However, two of the diastereoisomers were tested as minor components of mixtures and so their activity results are less meaningful for comparison.



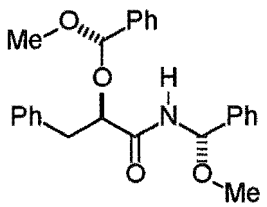
5.54



5.55



5.56



5.57

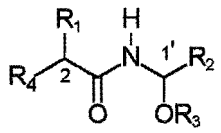
Table 6.4: IC_{50} values of unassigned adducts **5.54-5.57**

5.x	1′	1″	2	isomer[a]	IC_{50} (μg/cm^3)
5.54	*R*	*R*	*R*	A	52
5.55	*R*	*S*	*R*	B/A[b]	39
5.56	*S*	*R*	*R*	C/D[b]	24
5.57	*S*	*S*	*R*	D	28

a The four diastereoisomers A-D, corresponding to **5.54-5.57**, but are unassigned

b activity obtained on a 1:4 mixture of diastereoisomers

Conclusion



5.5

Structure to activity studies on the mycalamide skeleton of the class of natural products **5.1-5.4** have established the key features which were necessary or essential for the bioactivity observed across this class of natural products. These structural requirements have been summarized in structure **5.5**. Examples of general structure **5.5** have been synthesized and shown to give modest *in vitro* antitumour activity. The level of activity appears to be more sensitive to changes at R_2 than R_1 (structure **5.5**) and a (*1'R*)-configuration is favoured.

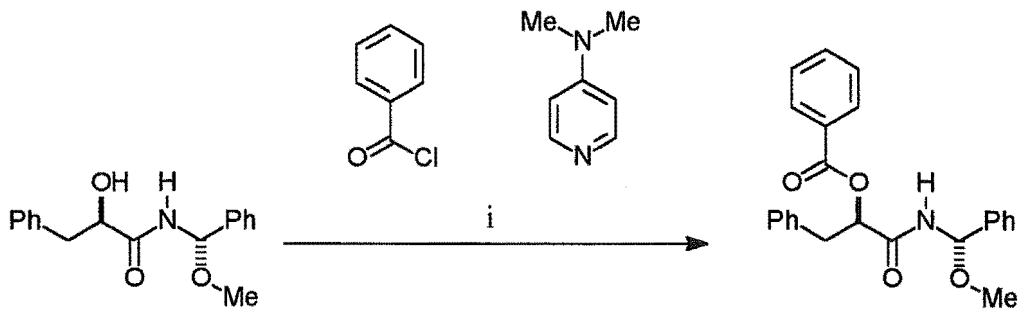
The proposal that mycalamide analogues may exploit the potent activity of the parents by acting as peptidomimetics was supported by the comparable or better activity of the peptide-based compounds (R_1 = NHR) over the R_1 = OR series. The conformationally restrained cyclic oxazolidinones also show comparable or better activity compared with the linear analogues. Their level of activity appears to favour a (*2R*)-configuration and to be insensitive to the (1')-configuration.

Chapter Seven

X-Ray Crystallography of an Unusual Crystal of DMAP/Benzoic Acid

During attempts at preparing a mycalamide analogue suitable for X-ray crystallographic analysis (see chapters five and six), crystals of (4-dimethylaminopyridine)$_5$(benzoic acid)$_3$(H_2O)$_{10}$ **7.1** (figures 7.1-7.3) were obtained accidentally, which had an unique structure and sparked further investigation.

The decahydrate **7.1** was obtained during the workup following the reaction of **5.46** (8 mg, 0.03 mmol) with benzoyl chloride (6 μL, 0.06 mmol), 4-dimethylaminopyridine (DMAP, 6 mg, 0.045 mmol) and triethylamine (7 μL, 0.06 mmol) in dichloromethane (2 cm^3) (scheme 7.1, see also scheme 5.16). After washing with aqueous $NaHCO_3$ the organic layer was dried and evaporated to give crude **5.58**. Recrystallization from diethyl ether-petroleum ether at 5 °C gave crystals of **7.1**.



5.46 **5.58**

Scheme 7.1: **i**, PhCOCl, DMAP, Et_3N, CH_2Cl_2, rt 2 d.

The following discussion of the crystal structure of **7.1** is the result of consultation with Professor Ward Robinson, Dr. Gautam Desiraju *et al.*.[138] The crystal structure of **7.1** was determined at 130 K and satisfactorily refined. There are eighteen residues in the asymmetric unit. Three out of the five DMAP residues are

protonated at the heterocyclic N atom and two of these form N–H···N hydrogen bonds with the two non-protonated DMAPs as shown in figure 7.1 [N(11)···N(21); N(31)···N(41)]. The third protonated DMAP forms a hydrogen bond [N(51)···O(62)] with a benzoate anion which is also linked to bridging water molecules, via hydrogen bonds O(61)···O(3) and O(62)···O(4). The three dimeric aggregates in figure 7.1 form a herringbone slab arrangement as shown in figure 7.2. These slabs alternate with benzoate hydrate layers which are shown in figure 7.3. Notice that the benzoate anions in figure 7.2 form the points of attachment of the slabs to the benzoate hydrate layers via O(3) and O(4) which lie within the layers. The planes of the molecules in the slabs and layers are, therefore, nearly perpendicular.

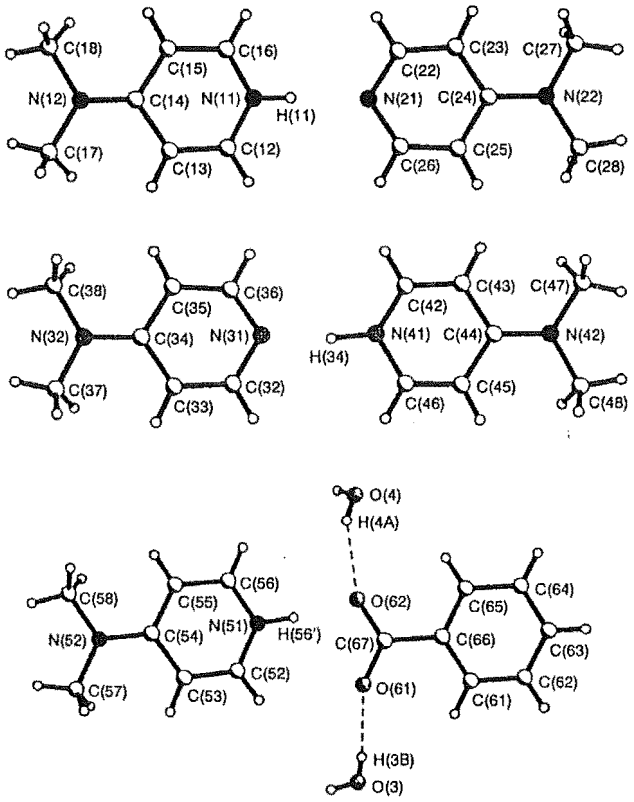


Figure 7.1: DMAP building blocks for the herringbone slabs in the crystal structure of **7.1**. The hydrogen bonds between benzoate anions and water molecules constitute the link between slabs and layers.

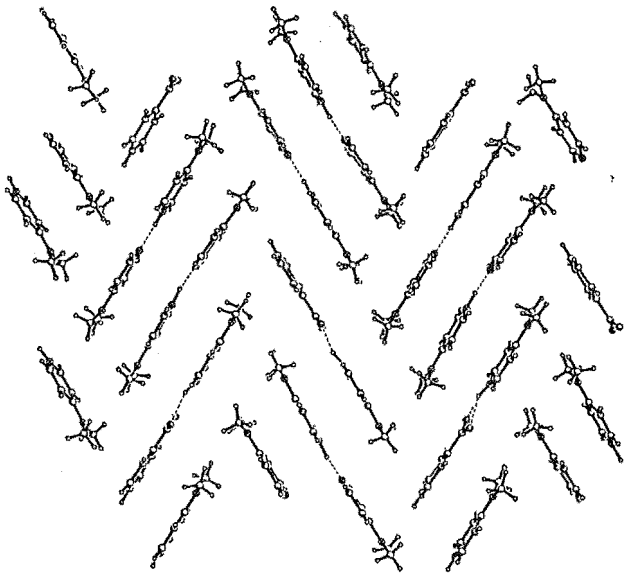


Figure 7.2: Herringbone slab arrangement in the crystal structure of **7.1**. [010] is vertical and [001] is horizontal.

The very high water content in this crystal occurs entirely within the benzoate layers which constitute the heart of this unique structure. Figure 7.3 shows that the benzoate anions are arranged with their hydrophobic portions facing each other forming a centrosymmetric dimer. This dimer is hydrophobic on the inside and hydrophilic on the outside. The outer hydrophilic surface is stabilized by a ring of water molecules and the hydrocarbon portions of the dimer are literally a drop of oil in the surrounding pool of water. Appropriately, the dimers are additionally stabilized by C-H···O hydrogen bonds to water molecules O(3F) and O(9A). In a sense, this structure is complementary to that of benzoic acid, wherein hydrogen-bonded centrosymmetric dimers are hydrophilic within and hydrophobic on the outside, being stabilized by herringbone contacts with other phenyl rings from neighbouring dimers. Alternatively, the structure in figure 7.3 may be likened to a two-dimensional equivalent of the polyhedral clathrate hydrate structures, in which a three-dimensional framework of water molecules surrounds the guest and the basic structural component is a regular pentagon formed by O-H···O hydrogen bonds. The regular solid generated by assembling such pentagons is the dodecahedron, but because space cannot be filled periodically with these dodecahedra, hexagons are incorporated, resulting in tetradeca-

, pentadeca- and hexadeca-hydra which alternate with the dodecahedra to generate the three-dimensional structure. In the two-dimensional benzoate hydrate layer seen in **7.1**, the polyhedra above reduce to quadrilaterals and other polygons formed by O-H···O hydrogen bonds. These include the parallelogram O(71K)-O(1E)-O(71I)-O(1G), the quadrilateral O(72I)-O(7A)-O(3A)-O(9E) and the distorted pentagon O(1G)-O(71I)-O(72I)-O(9E)-O(5G). Different polygons are required for space filling in the three-dimensional clathrate hydrates. Interestingly, one or two benzoate O atoms are included in each of these polygons as in the three-dimensional polyhedral clathrate structure of tetra(butyl)ammonium benzoate hydrate, which seems to be the only reported crystal structure[145,146] even remotely resembling **7.1**.

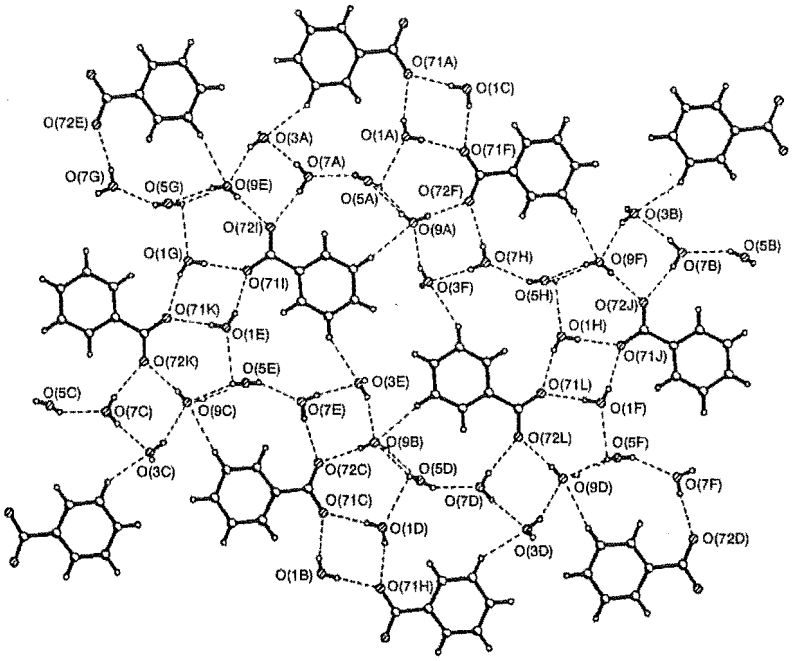


Figure 7.3: Hydrated benzoate layer in the crystal structure of **7.1**. The layers are parallel to [011] and symmetry-independent, although geometrically identical layers may be found at $x = 0$ and at $x = 0.5$. Symmetry-related atoms have the same numerical code. [001] is along the translation O(71K)···O(71L) and [010] is perpendicular to this direction. Notice that the centrosymmetric benzoate dimer is completely encircled by water molecules.

There are other unusual features in the crystal structure of **7.1**: 1) The herringbone slabs consist of twice the number of cations as they do anions while the layers consist of anions only. There are, in effect, fairly large concentrations of charge in different regions of the structure, and anions and cations do not tend to make a maximum number of close contacts as would be expected in a salt. A possible reason for this is the presence of a large amount of water in the crystal which, with its high dielectric constant, may disperse the charge effectively; 2) The two hydrated layers which sandwich each herringbone slab are crystallographically independent though they are, geometrically speaking, virtually identical; 3) The presence of eighteen residues (five DMAPs, three benzoates and ten water molecules) in the asymmetric unit is unprecedented for a small-molecule structure.

It is pertinent to mention that all subsequent attempts to crystallize **7.1** from solutions of DMAP and benzoic acid, proved futile. Rather, two simpler crystals, a DMAP benzoate monohydrate, mp 101-103 °C, and an anhydrous acid benzoate, $(DMAP)(PhCO_2H)_2$, mp 128-129 °C, were obtained. The crystal structures of these substances were also determined, but their packing patterns proved to be straightforward. It is possible that the highly unusual crystal structure of **7.1** is a result of low concentrations of benzoate anion formed by hydrolysis of PhCOCl by $NaHCO_3$ in the workup. If so, it may be expected that polymorphic or related crystalline forms of other substances may also be obtained by generating the relevant material(s) *in situ*. It is well known that crystals of better quality are obtained when low concentrations of material are generated electrochemically.[147] That low concentrations are important in crystal growth is also shown by the efficacy of the gel diffusion method in obtaining crystals of organic donor-acceptor complexes.[148] The present example shows that entirely different and novel crystalline forms may be obtained when the crystallization conditions are such that low concentrations of material are generated chemically.

Even with the recent advances in experimental and computational crystal engineering, it would be impossible to predict the details of this crystal structure, from such simple constituents as DMAP, benzoic acid and water.

X-ray Crystallographic Determination for 7.1

$C_{56}H_{88}N_{10}O_{16}$, M = 1157.36, monoclinic space group $P2_1/c$, a = 18.055(8), b = 19.964(5), c = 17.198(2) Å, β = 99.27(5)°, V = 6118(3) Å^3, D_c = 1.26 g gm^{-3}, Z = 4, crystal size 0.70 0.42 0.20 mm, μ(Cu-Kα) = 0.764 mm^{-1}, F(000) = 2488, T = 130(2) K, Siemens P4 diffractometer, ω scans, 6494 reflections collected, 3148 onserved at 2σ level, structure solution with SHELXS-86, refinement with SHELXL-93 on F^2 for 808 parameters, non-H atoms anisotropic, H-atoms in calculated positions. R = 0.0951, wR = 0.2164, Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

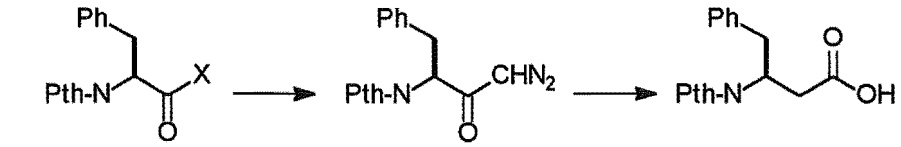
Experimental

cis-Hydroxyethylamine Isostere

General procedure

Mps were taken using a Reichert hot-stage microscope and are uncorrected. Optical rotations were measured on a JASCO J-20C recording spectropolarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}, with the concentration given in units of g cm^{-3}. IR spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either a Varian CFT300 or XT300 spectrometer. Samples were run in $CDCl_3$ solution, with Me_4Si as an internal standard, unless otherwise stated. Mass spectra were obtained using a Kratos MS80RFA spectrometer. Radial chromatography was performed on a Chromatatron (Harrison and Harrison) using Merck type 60 PF254 silica gel. Compounds **3.4**, **3.8**, *L*-Ala-OBzl, Cbz-*L*-Asn and *L*-Asn-O^{t}Bu were commercially available.

Preparation[102,104] of (*S*)-4-phenyl-3-(*N*-phthalylamino)butanoic acid 3.7



3.4 X = OH
3.5 X = Cl

3.6

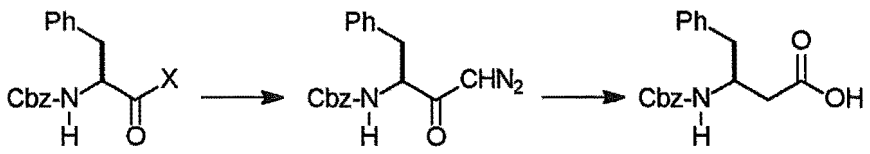
3.7

To a solution of Pth-*L*-Phe **3.4** (100 mg, 0.34 mmol) in dry benzene (5 cm^3) was added DMF (20 μL) and oxalyl chloride (0.16 cm^3, 1.70 mmol) and the mixture was stirred at rt for 1 h. The solution was evaporated to give the acid chloride **3.5** which was used without further purification; ^1H NMR, δ 3.42 (1H, dd, *J* 14.2 & 10.8, CH_APh), 3.48 (1H, dd, *J* 14.2 & 5.3, CH_BPh), 5.21 (1H, dd, *J* 10.8 & 5.3, $CHCH_2$), 7.01-7.10 (5H, m, ArH), 7.62 (2H, m, PthH), 7.71 (2H, m, PthH); IR /cm^{-1} 1772, 1750. The acid chloride **3.5** was redissolved in dry ether (5 cm^3) and to it was added a

dried (Na_2SO_4) solution of diazomethane (5 mmol) in ether (15 cm^3). The mixture was left to stand at 5 °C for 18 h and evaporated. Recrystallization from benzene-hexane gave the α-diazoketone **3.6** (102 mg, 93%); ^1H NMR, δ 3.54 (2H, m, CH_2Ph), 5.09 (1H, dd, *J* 10.3 & 6.4, C*H*CH_2), 5.38 (1H, s, CHN_2), 7.15 (5H, m, ArH), 7.71 (2H, m, PthH), 7.79 (2H, m, PthH); ^{13}C NMR, δ 33.92 (CH_2Ph), 54.25 (CHN_2), 58.33 (*C*HCH_2), 123.55, 126.88, 128.61, 128.85 & 134.32 (ArCH), 131.39 & 136.69 (ArC), 167.59 (PthCO), 189.32 (PheCO); IR /cm^{-1} 2098 (CHN_2), 1708, 1651.

To a stirred suspension of freshly prepared silver(I) oxide (61 mg, 0.26 mmol), Na_2CO_3 (31 mg, 0.29 mmol) and $Na_2S_2O_3.5H_2O$ (25 mg, 0.10 mmol) in water (2 cm^3) was added dropwise, at 50-60 °C, a solution of the α-diazoketone **3.6** (83 mg, 0.26 mmol) in dioxane (4 cm^3). The mixture was refluxed for 2 h, cooled, diluted with water and filtered. The solution was acidified with aqueous 10% HNO_3, extracted with ether (3 x 5 cm^3), dried and evaporated to give the acid **3.7** (52 mg, 65%); (Found: M^+ 309.1008. $C_{18}H_{15}NO_4$ requires *m/z* 309.1001); ^1H NMR, δ 2.87 (1H, dd, *J* 16.6 & 5.4, CH_2Ph or CH_2CO), 3.13 (1H, dd, *J* 13.7 & 6.4, CH_2Ph or CH_2CO), 3.24 (2H, m, CH_2Ph or CH_2CO), 4.91 (1H, m, C*H*CH_2), 7.17 (5H, m, ArH), 7.66 (2H, m, PthH), 7.73 (2H, m, PthH); ^{13}C NMR, δ 35.94 & 38.37 (CH_2Ph & *C*H_2CO), 48.87 (*C*HCH_2), 123.20, 126.80, 128.50, 128.96 & 133.88 (ArCH), 131.47 & 136.99 (ArC), 168.03 (PthCO), 175.95 (CO_2H); IR /cm^{-1} 3032, 1773, 1708.

Preparation[104] of (*S*)-3-(*N*-benzyloxycarbonylamino)-4-phenylbutanoic acid 3.11



3.8 X = OH
3.9 X = OCO_2Et

3.10 **3.11**

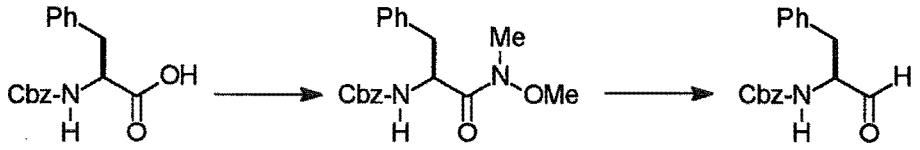
To a stirred solution of Cbz-*L*-Phe **3.8** (4.999 g, 16.70 mmol) in dry ether-THF (60 cm^3 of a 1:1 mixture) was added triethylamine (2.33 mL, 16.70 mmol) and ethyl chloroformate (1.60 mL, 16.70 mmol) at -20 °C. After 15 min at -20 °C the mixed anhydride **3.9** was added to a dried (Na_2SO_4) solution of diazomethane (33 mmol) in

ether (50 cm^3). The mixture was stirred at -10 °C for 1 h and then left to stand at 5 °C for 18 h. The mixture was washed with ice-cooled saturated aqueous $NaHCO_3$ (10 cm^3), water (10 cm^3), dried and evaporated to give the α-diazoketone[104] **3.10** as a yellow solid (5.01 g, 93%); [Found: $(M-N_2)^+$ 295.1206. $C_{18}H_{17}NO_3$ requires *m/z* 295.1208]; ^1H NMR, δ 3.05 (2H, d, *J* 6.8, CH_2Ph), 4.48 (1H, m, C*H*CH_2), 5.08 (2H, s, CbzCH_2), 5.20 (1H, s, CHN_2), 5.34 (1H, d, NH), 7.16-7.39 (10H, m, ArH); ^{13}C NMR, δ 38.30 (CH*C*H_2), 54.53 (CHN_2), 58.76 (*C*HCH_2), 66.91 (CbzCH_2), 126.97, 127.93, 128.12, 128.44, 128.57 & 129.21 (ArCH), 135.94 & 136.05 (ArC), 155.66 (CbzCO), 192.78 (PheCO); IR /cm^{-1} 2124 (CHN_2), 1730, 1635.

To a stirred suspension of freshly prepared silver(I) oxide (3.59 g, 1 equiv), Na_2CO_3 (0.74 g, 0.45 equiv) and $Na_2S_2O_3.5H_2O$ (1.54 g, 0.4 equiv) in water (50 cm^3) was added dropwise at 50-60 °C, a solution of the α-diazoketone **3.10** (5.01g, 15.53 mmol) in dioxane (50 cm^3). The mixture was stirred at 78-85 °C for 1.5 h, cooled, diluted with water and filtered. The solution was acidified with aqueous 10% HNO_3, extracted with ether (3 x 20 cm^3), dried and evaporated to give the acid **3.11** (2.996 g, 62%); (Found: M^+ 313.1313. $C_{18}H_{19}NO_4$ requires *m/z* 313.1314); mp 119-121 °C (literature[104] mp 118-119 °C); $[\alpha]_D^{20}$ -20° (c 0.023 in CH_2Cl_2); ^1H NMR, δ 2.58 (2H, m, CH_2CO), 2.88 (1H, dd, *J* 13.7 & 7.5, CH_APh), 2.97 (1H, dd, *J* 13.6 & 6.4, CH_BPh), 4.25 (1H, m, C*H*CH_2), 5.08 (2H, s, CbzCH_2), 5.28 (1H, d, NH), 7.17-7.38 (10H, m, ArH); ^{13}C NMR, δ 37.19 (*C*H_2CO), 40.07 (CH_2Ph), 49.13 (*C*HCH_2), 66.73 (CbzCH_2), 126.70, 128.01, 128.09, 128.46, 128.56 & 129.29 (ArCH), 137.27 (ArC), 155.74 (CbzCO), 176.64 (CO_2H).

Preparation of *N*-benzyloxycarbonyl-*L*-phenylalaninal 3.16

Method A[107]

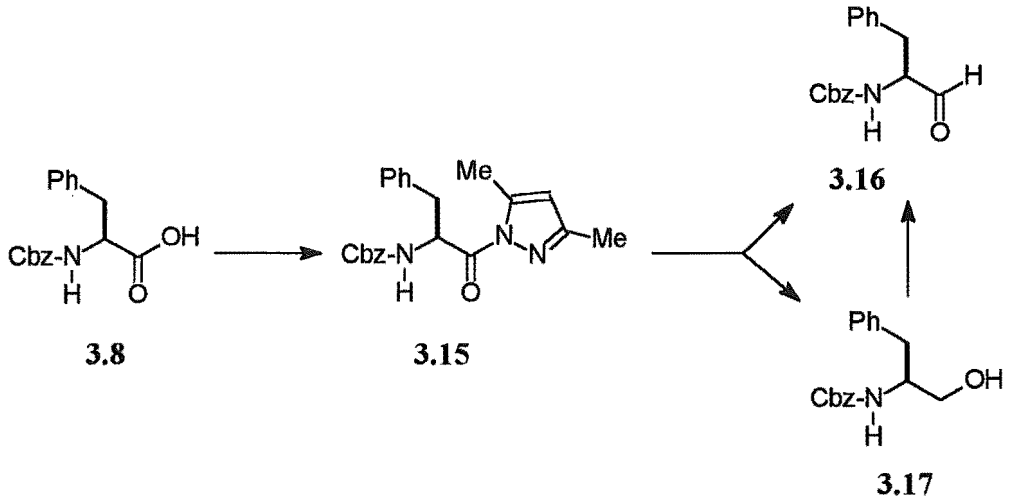


3.8 **3.14** **3.16**

To a solution of Cbz-*L*-Phe **3.8** (501 mg, 1.67 mmol) in dichloromethane (5 cm^3) was added triethylamine (0.233 cm^3, 1.67 mmol) and BOP (739 mg, 1.67 mmol) and the mixture was stirred at rt for 5 min. *O,N*-Dimethylhydroxylamine hydrochloride (180 mg, 1.84 mmol) and triethylamine (0.256 cm^3, 1.84 mmol) were added and stirring was continued at rt for a further 8 h. The mixture was washed with aqueous 2M HCl (5 cm^3), saturated aqueous $NaHCO_3$ (5 cm^3) and saturated aqueous NaCl (5 cm^3), dried and evaporated. Purification of the crude product by flash chromatography eluting with ethyl acetate-dichloromethane (1:3) gave **3.14** (494 mg, 86%); ^{1}H NMR, δ 2.91 (1H, dd, *J* 13.7 & 7.3, CHCH_APh), 3.08 (1H, dd, *J* 13.7 & 5.8, CHCH_BPh), 3.18 (3H, s, NCH_3), 3.68 (3H, s, OCH_3), 5.02 (1H, m, C*H*CH_2), 5.03 & 5.09 (2H, ABq, *J* 12.2, CbzCH_2), 5.41 (1H, d, *J* 7.8, NH), 7.15 (2H, m, ArH), 7.24-7.37 (8H, m, ArH).

To a suspension of lithium aluminium hydride (102 mg, 2.67 mmol) in dry THF (5 cm^3) was added dropwise at -50 °C over 5 min, a solution of **3.14** (763 mg, 2.23 mmol) in THF (15 cm^3). After stirring at -50 °C for 10 min, the mixture was allowed to warm to 0 °C over 20 min before recooling to -50 °C. Ethyl acetate (2 cm^3) was slowly added to decompose any remaining lithium aluminium hydride and the mixture was stirred at 0 °C for 20 min. The mixture was filtered and evaporated. The residue was redissolved in ethyl acetate (10 cm^3), washed with ice-cooled aqueous 1M HCl (5 cm^3), aqueous 10% $NaHCO_3$ (5 cm^3) and water (5 cm^3), dried and evaporated to give the aldehyde[113] **3.16** (484 mg, 77%); ^{1}H NMR, δ 3.14 (2H, d, *J* 6.4, CHCH_2), 4.51 (1H, m, C*H*CH_2), 5.11 (2H, s, CbzCH_2), 5.33 (1H, br d, *J* 6.3, NH), 7.14 (2H, m, ArH), 7.24-7.37 (8H, m, ArH), 9.64 (1H, s, CHO); ^{13}C NMR, δ 35.36 (C*H*CH_2), 61.04 (*C*HCH_2), 67.08 (CbzCH_2), 127.18, 128.13, 128.26, 128.54, 128.81 & 128.29 (ArCH), 135.38 (ArC), 198.80 (CHO).

Method B[113]

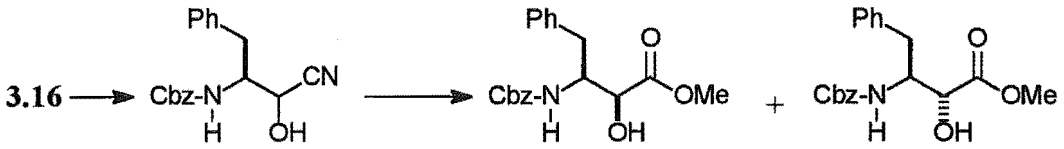


To a solution of Cbz-*L*-Phe **3.8** (5.066 g, 16.90 mmol) and 3,5-dimethylpyrazole (1.936 g, 20.30 mmol) in chloroform (250 cm^3) at -10 °C was added dicylcohexylcarbodiimide (DCC, 3.492 g, 20.30 mmol) and the mixture was stirred for 1 h before warming to rt and stirring for a further 2 d. After removal of the dicyclohexylurea byproduct, the solvent was evaporated. The residue was redissolved in ethyl acetate (50 cm^3), washed with aqueous 2M HCl (10 cm^3) and water (10 cm^3), dried and evaporated to give a crude residue which was recrystallized from ethyl acetate-petroleum ether to give fine white needles of **3.15** (5.443 g, 85%); mp 136-138 °C (literature[113] mp 142 °C); ^{1}H NMR, δ 2.27 (3H, s, CH_3), 2.48 (3H, s, CH_3), 3.09 (1H, dd, *J* 13.8 & 7.1, CHCH_APh), 3.33 (1H, dd, *J* 13.7 & 4.9, CHCH_BPh), 5.05 & 5.10 (2H, ABq, *J* 12.2, CbzCH_2), 5.47 (1H, d, *J* 8.8, NH), 5.58 (1H, m, C*H*CH_2), 5.99 (1H, s, C*H*CMe), 7.06 (2H, m, ArH), 7.22-7.33 (8H, m, ArH).

Reduction of **3.15** (5.443 g, 14.40 mmol) was carried out as above to give an oily mixture (3.183 g, 5:3 by ^{1}H NMR) of the aldehyde **3.16** (49%) and *N-benzyloxycarbonyl-L-phenylalaninol* **3.17** (29%); ^{1}H NMR, δ 2.86 (2H, d, *J* 7.3, CHCH_2Ph), 3.58 (1H, dd, *J* 11.2 & 4.9, CH_AOH), 3.68 (1H, dd, *J* 11.2 & 3.9, CH_BOH), 3.94 (1H, m, C*H*CH_2), 5.00 (1H, d, *J* 7.3, NH), 5.07 (2H, s, CbzCH_2), 7.18-7.39 (10H, m, ArH).

To a stirred solution of Dess-Martin periodinane[108] (774 mg, 1.82 mmol) in dichloromethane (5 cm^3) was added a solution of the alcohol **3.17** (476 mg, 1.67 mmol) in dichloromethane (5 cm^3) and the mixture was stirred at rt for 1 h. Ether (25 cm^3) and a solution of $Na_2S_2O_3.5H_2O$ (2 g, 8 mmol) in saturated aqueous $NaHCO_3$ (20 cm^3) were added and the mixture was stirred at rt for 15 min. The organic layer was washed with saturated aqueous $NaHCO_3$ (5 cm^3), water (5 cm^3) and saturated aqueous NaCl (5 cm^3), dried and evaporated to give further aldehyde **3.16** (468 mg, 98%).

Preparation of (*2S,3S*)- and (*2R,3S*)-3-(*N*-benzyloxycarbonylamino)-2-hydroxy-4-phenylbutanoic acid methyl esters[106] 3.19 and 3.20



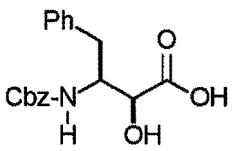
3.18 **3.19** **3.20**

To a solution of **3.16** (484 mg, 1.72 mmol) in ethyl acetate (10 cm^3) was added a solution of potassium cyanide (143 mg, 1.72 mmol) in water (10 cm^3) and the mixture was stirred vigorously at rt for 18 h. The organic layer was washed with water (5 cm^3), dried and evaporated to give an epimeric mixture (1:1 by ^1H NMR) of the cyanohydrins **3.18** (408 mg, 76%); ^1H NMR, epimer A δ 2.85 (1H, m, $CHCH_APh$), 3.05 (1H, dd, *J* 14.2 & 6.3, $CHCH_BPh$), 3.96 (1H, m, $CHCH_2$), 4.49 (1H, d, *J* 3.9, CHOH), 5.01 (2H, s, $CbzCH_2$), 5.18 (1H, d, *J* 7.9, NH), 7.10-7.31 (10H, m, ArH), epimer B δ 2.85 (2H, d, *J* 7.8, $CHCH_2$), 4.17 (1H, m, $CHCH_2$), 4.42 (1H, d, *J* 3.0, CHOH), 5.00 (2H, s, $CbzCH_2$), 5.08 (1H, d, *J* 8.8, NH); IR /cm^{-1} 3431 (OH), 2258, 2253 (CN).

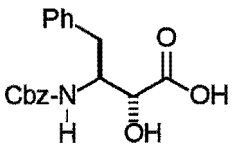
The mixture of **3.18** (1.781g, 5.74 mmol) was dissolved in dry and pre-cooled ether-methanol (80 cm^3 of a 3:1 mixture), previously saturated with gaseous hydrogen chloride. The mixture was stirred at 5 °C for 24 h, ice-cooled water (15 cm^3) was added (keeping the temperature below 10 °C) and stirring was continued at 5 °C for 2 d. The mixture was concentrated and extracted with dichloromethane (3 x 10 cm^3). The combined organic extracts were washed with water (5 cm^3) and brine (5 cm^3),

dried and evaporated to give a mixture of the methyl esters **3.19** and **3.20**. Purification by flash chromatography, eluting with ethyl acetate-hexane (1:5 to 3:5) gave **3.20** (567 mg, 29%); (Found: M^+ 343.1415. $C_{19}H_{21}NO_5$ requires *m/z* 343.1419); mp 84-87 °C; $[\alpha]_D^{20}$ -45° (c 0.032 in CH_2Cl_2); R_F 0.31 (3:7 EtOAc-pet. ether); ^{1}H NMR[106], δ 2.92 (2H, m, CHC*H*$_2$), 3.65 (3H, s, OCH_3), 4.09 (1H, d, *J* 1.5, C*H*OH), 4.33 (1H, m, C*H*CH_2), 5.01 (2H, s, $CbzCH_2$), 5.43 (1H, d, *J* 9.8, NH), 7.19-7.30 (10H, m, ArH); ^{13}C NMR, δ 37.96 ($CHCH_2$), 52.54 (OMe), 54.70 ($CHCH_2$), 66.49 ($CbzCH_2$), 70.08 (CHOH), 126.48, 127.68, 127.82, 128.24, 128.38 & 129.19 (ArCH), 136.24 & 137.21 (ArC), 155.66 (CbzCO), 173.91 (CO). Further elution gave **3.19** (584 mg, 30%); (Found: M^+ 343.1410. $C_{19}H_{21}NO_5$ requires *m/z* 343.1419); mp 116-117 °C; $[\alpha]_D^{20}$ -5° (c 0.02 in MeOH); R_F 0.21 (3:7 EtOAc-pet. ether); ^{1}H NMR[106], δ 2.80 (2H, m, CHC*H*$_2$), 3.56 (3H, s, OCH_3), 4.34 (1H, d, *J* 3.4, C*H*OH), 4.38 (1H, m, C*H*CH_2), 5.05 (2H, s, $CbzCH_2$), 5.15 (1H, d, *J* 9.2, NH), 7.18-7.36 (10H, m, ArH); ^{13}C NMR, δ 35.37 ($CHCH_2$), 52.30 (OMe), 54.54 ($CHCH_2$), 66.51 ($CbzCH_2$), 72.09 (CHOH), 126.43, 127.78, 127.86, 128.16, 128.26 & 129.26 (ArCH), 136.19 & 136.83 (ArC), 155.80 (CbzCO), 172.76 (CO).

Preparation of (*2S,3S*)- and (*2R,3S*)-3-(*N*-benzyloxycarbonylamino)-2-hydroxy-4-phenylbutanoic acids[106] 3.21 and 3.23



3.21

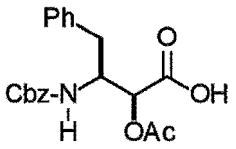


3.23

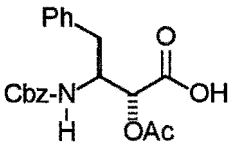
The methyl ester **3.19** or **3.20** (1 equiv) and sodium hydroxide (1.4 equiv) was dissolved in methanol-water (0.5 to 5 cm^3 of a 4:1 mixture) and the solution was stirred at rt for 2 h. The methanol was evaporated and the aqueous layer acidified (pH<3 by universal indicator paper) with aqueous 2M HCl, precipitating a white solid. The solid was extracted into ethyl acetate (3 x 10 cm^3), dried and evaporated to give the corresponding free acid **3.21** (88%) or **3.23** (88%). Acid **3.21**; ^{1}H NMR[106]

($CDCl_3$, d_6-DMSO), δ 2.81 (2H, m, CHC*H*$_2$), 4.30 (2H, m, C*H*OH & C*H*CH_2), 4.98 (2H, s, CbzCH_2), 6.07 (1H, d, *J* 9.2, NH), 7.12-7.35 (10H, m, ArH). Acid **3.23**; ^{1}H NMR[106] ($CDCl_3$, d_6-DMSO), δ 2.92 (2H, m, CHC*H*$_2$), 4.01 (1H, d, *J* 1.9, C*H*OH), 4.27 (1H, m, C*H*CH_2), 5.02 (2H, s, CbzCH_2), 6.25 (1H, d, *J* 9.8, NH), 7.20-7.36 (10H, m, ArH).

Preparation of (*2S,3S*)- and (*2R,3S*)-2-acetoxy-3-(*N*-benzyloxycarbonylamino)-4-phenylbutanoic acids 3.22 and 3.24



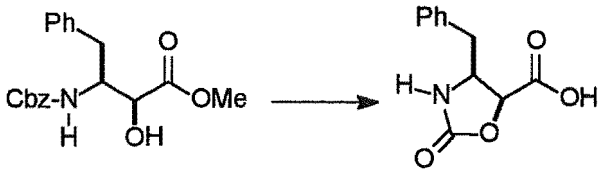
3.22



3.24

The acid **3.21** (609 mg, 1.85 mmol) or **3.23** (306 mg, 0.93 mmol) was dissolved in pyridine (3 cm^3), acetic anhydride (3 equiv) was added and the mixture was stirred at rt for 18 h. Saturated aqueous NaCl (3 cm^3) was added and the mixture was extracted with chloroform (4 x 10 cm^3), dried and evaporated to give the corresponding acetate **3.22** or **3.24** (quant) plus traces of pyridine. Acetate **3.22**; $[\alpha]_D^{20}$ -6° (c 0.009 in CH_2Cl_2); ^{1}H NMR, δ 2.00 (3H, s, CH_3), 2.72 (1H, m, CHCH_APh), 2.99 (1H, m, CHCH_BPh), 4.43 (1H, m, C*H*CH_2), 4.77 & 4.95 (2H, ABq, *J* 12.2, CbzCH_2), 4.99 (1H, d, *J* 5.8, C*H*OAc), 5.54, (1H, d, *J* 8.8, NH), 7.06-7.26 (10H, m, ArH); ^{13}C NMR, δ 21.54 (Me), 36.27 (CHCH_2), 53.19 (*C*HCH_2), 66.52 (CbzCH_2), 77.10 (CHOAc), 126.30, 127.67, 127.79, 128.28 & 129.19 (ArCH), 136.30 & 137.64 (ArC), 156.38 (CbzCO), 172.01 & 174.48 (CO); IR /cm^{-1} 1750, 1713, 1626. Acetate **3.24**; $[\alpha]_D^{20}$ -18° (c 0.018 in CH_2Cl_2); ^{1}H NMR, δ 2.19 (3H, s, CH_3), 2.84 (1H, dd, *J* 13.2 & 8.3, CHCH_APh), 2.96 (1H, dd, *J* 13.4 & 7.0, CHCH_BPh), 4.59 (1H, m, C*H*CH_2), 5.03 (1H, d, *J* 5.8, CHOAc), 5.00 & 5.09 (2H, ABq, *J* 12.7, CbzCH_2), 5.32, (1H, d, *J* 9.7, NH), 7.18-7.32 (10H, m, ArH); IR /cm^{-1} 1750, 1713, 1628.

Preparation of (*4S,5S*)-4-benzyl-2-oxo-5-oxazolidinecarboxylic acid[106] 3.25



3.19 **3.25**

The methyl ester **3.19** (31 mg, 0.09 mmol) and potassium carbonate (27 mg, 0.18 mmol) were dissolved in methanol-water (3 cm^3 of a 9:1 mixture) and stirred at rt for 18 h. The methanol was evaporated and the aqueous layer was acidified (pH<3) with aqueous 2M HCl, precipitating a white solid. The solid was extracted into ethyl acetate (3 x 5 cm^3), dried and evaporated to give the acid **3.25** (14 mg, 72%); ^1H NMR[106] ($CDCl_3$, d_6-DMSO), δ 2.64 (1H, dd, *J* 13.7 & 9.7, CH_APh), 2.98 (1H, dd, *J* 13.7 & 3.9, CH_BPh), 4.31 (1H, m, CHN), 5.05 (1H, d, *J* 8.8, CHO), 7.19-7.34 (5H, m, ArH); ^{13}C NMR ($CDCl_3$, d_6-DMSO), δ 36.09 (CH_2Ph), 53.83 (CHN), 74.75 (CHO), 125.58, 127.42 & 127.98 (ArCH), 135.44 (ArC), 156.48 (NCO), 172.98 (CO_2H).

Preparation of compounds 3.32, 3.34, 3.36, 3.37, 3.39, 3.41 and 3.42

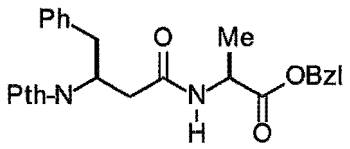
General Coupling Method A (DCC/HOBT)

To a solution of the carboxylic acid (1 equiv) in dichloromethane (5 cm^3/mmol) was added at 0 °C, triethylamine (1 equiv), HOBT (1 equiv) and the amine (1 equiv; alternatively, 1 equiv of the amine.HCl or HBr salt and an extra 1 equiv of triethylamine), and the mixture was stirred for 10 min. DCC (1 equiv) was added, and stirring was continued at 0 °C for a further 10 min and then at rt for 18 h. The mixture was filtered and evaporated. The residue was redissolved in ethyl acetate (5 cm^3), washed with aqueous 2M HCl (2.5 cm^3), aqueous 10% $NaHCO_3$ (2.5 cm^3) and water (2.5 cm^3), dried and evaporated. The crude product was purified by flash chromatography or recrystallization to remove any remaining dicyclohexylurea.

General Coupling Method B (BOP)

To a solution of the carboxylic acid (1 equiv), amine (1 equiv; alternatively, 1 equiv of the amine.HCl or HBr salt and an extra 1 equiv of triethylamine) and BOP (1.1 equiv) in dichloromethane or DMF (0.5 to 5 cm^3) was added triethylamine (2 equiv) and the mixture was stirred at rt for 1 h. A further portion of triethylamine (1 equiv) was added and stirring was continued at rt for 18 h. Saturated aqueous NaCl (3 cm^3) was added and the mixture extracted with ethyl acetate (3 x 5 cm^3), washed with aqueous 2M HCl (2.5 cm^3), aqueous 1M $NaHCO_3$ (3 x 2.5 cm^3) and water (2.5 cm^3), dried and evaporated. The product was purified by flash chromatography.

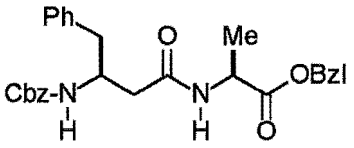
Benzyl (*2S*)-2-[(*3S*)-4-phenyl-3-(*N*-phthalylamino)butanoylamino]propanoate 3.32



3.32

Pth-*L*-βPhe **3.7** (55 mg, 0.18 mmol) was reacted with *L*-alanine benzyl ester hydrochloride according to general coupling method A. Purification by flash chromatography eluting with a gradient of ethyl acetate-dichloromethane (1:100 to 1:10) gave the amide **3.32** (38 mg, 45%); (Found: M^+ 470.1839. $C_{28}H_{26}N_2O_5$ requires *m/z* 470.1841); R_F 0.38 (1:20 EtOAc-CH_2Cl_2); ^1H NMR, δ 1.32 (3H, d, *J* 6.9, CH_3), 2.75 (1H, dd, *J* 14.6 & 5.4, CHC*H*$_2$Ph or CH_2CO), 3.14 (2H, m, CHC*H*$_2$Ph or CH_2CO), 3.26 (1H, dd, *J* 12.2 & 9.2, CHC*H*$_2$Ph or CH_2CO), 4.53 (1H, m, C*H*Me), 4.99 (3H, m, C*H*CH_2 & BzlCH_2), 6.16 (1H, d, *J* 7.3, NH), 7.13-7.33 (10H, m, ArH), 7.65 (2H, m, PthH), 7.72 (2H, m, PthH); ^{13}C NMR, δ 18.16 (Me), 38.29 & 38.53 (CH*C*H_2Ph & CH_2CO), 48.01 & 49.73 (*C*HMe & *C*HCH_2), 66.93 (BzlCH_2), 123.11, 126.74, 128.00, 128.34, 128.50, 128.54, 129.01 & 133.72 (ArCH), 131.69 & 137.23 (ArC), 168.29 (PthCO), 169.30 & 172.50 (CO).

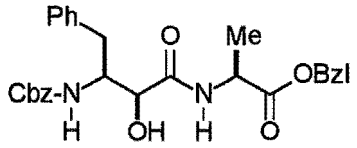
Benzyl (2*S*)-2-[(3*S*)-4-phenyl-3-(*N*-benzyloxycarbonylamino)butanoylamino]-propanoate 3.34



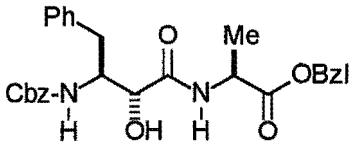
3.34

Cbz-*L*-βPhe **3.11** (1.451 g, 4.6 mmol) was reacted with *L*-alanine benzyl ester hydrochloride according to general coupling method A. Crystallization of the crude product from ethyl acetate-petroleum ether gave the amide **3.34** as fine white crystals (1.025 g, 47%); [Found: $(M\text{-}PhCH_2)^+$ 383.1606. $C_{21}H_{23}N_2O_5$ requires *m/z* 383.1607]; mp 144-145 °C; $[\alpha]_D^{20}$ -19° (c 0.041 in CH_2Cl_2); ^1H NMR, δ 1.37 (3H, d, *J* 7.4, CH_3), 2.33 (1H, dd, *J* 15.2 & 5.4, $CHCH_ACO$), 2.45 (1H, dd, *J* 14.8 & 5.2, $CHCH_BCO$), 2.80 (1H, dd, *J* 13.4 & 8.1, $CHCH_APh$), 2.99 (1H, dd, *J* 13.7 & 6.4, $CHCH_BPh$), 4.14 (1H, m, $CHCH_2$), 4.61 (1H, m, CHMe), 5.07 (2H, s, $CbzCH_2$), 5.16 & 5.22 (2H, ABq, *J* 12.2, $BzlCH_2$), 5.75 (1H, d, *J* 7.8, NHCHMe, 6.09 (1H, d, *J* 5.9, NHCbz), 7.19-7.40 (15H, m, ArH); ^{13}C NMR, δ 18.10 (Me), 38.43 (CH_2CO), 40.13 ($CHCH_2Ph$), 48.07 (CHMe), 50.11 ($CHCH_2$), 66.48 ($CbzCH_2$), 67.19 ($BzlCH_2$), 126.57, 127.90, 127.97, 128.17, 128.44, 128.46, 128.56, 128.62 & 129.29 (ArCH), 135.25 & 137.88 (ArC), 155.86 (CbzCO), 170.41, 172.60 (CO); IR /cm^{-1} 3427, 3030, 1713, 1670, 1499.

Benzyl (*2S*)-2-[(*2S,3S*)- and (*2S*)-2-[(*2R,3S*)-3-(*N*-benzyloxycarbonylamino)-2-hydroxy-4-phenyl-butanoylamino]propanoate 3.36 and 3.37



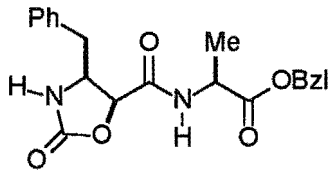
3.36



3.37

A mixture of the acids **3.23** and **3.21** (4:1 by ^{1}H NMR) (45 mg, 0.14 mmol) was reacted with *L*-alanine benzyl ester hydrochloride according to general coupling method A. Purification by flash chromatography eluting with ethyl acetate-dichloromethane (1:9 to 1:3) gave a mixture (42 mg, 61% yield, 4:1 by ^{1}H NMR) of the amides **3.37** and **3.36**; [Found: $(M\text{-}PhCH_2)^+$ 399.1556. $C_{21}H_{23}N_2O_6$ requires *m/z* 399.1556]; ^{1}H NMR, **3.37** (from the mixture) δ 1.35 (3H, d, *J* 7.3, CH_3), 2.99 (2H, m, $CHCH_2Ph$), 4.13 (2H, m, $CHCH_2$ & $CHOH$), 4.57 (1H, m, $CHMe$), 4.99 (2H, s, $CbzCH_2$), 5.06 & 5.15 (2H, ABq, *J* 12.2, $BzlCH_2$), 5.20 (1H, d, *J* 5.4, OH), 5.55 (1H, d, *J* 8.8, CbzNH), 7.17-7.36 (15H, m, ArH), **3.36** (from the mixture) δ 1.29 (3H, d, *J* 7.4, CH_3); ^{13}C NMR, **3.36/3.37** δ 17.69/17.88 (Me), 36.42 ($CHCH_2Ph$), 47.74/47.80 ($CHMe$), 55.37/55.55 ($CHCH_2$), 66.69/66.74/66.94/67.02 ($CbzCH_2$ & $BzlCH_2$), 72.61/72.43 (CHOH), 126.39, 127.67, 127.78, 127.94, 128.28, 128.32, 128.46 & 129.18 (ArCH), 135.18, 136.16, 137.71 & 137.65 (ArC), 156.78 & 157.05 (CbzCO), 172.16 & 172.39 (BzlCO).

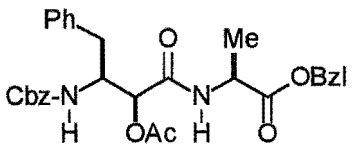
Benzyl (*2S*)-2-[(*4S,5S*)-4-benzyl-2-oxooxazolidin-5-ylcarbonylamino]propanoate 3.39



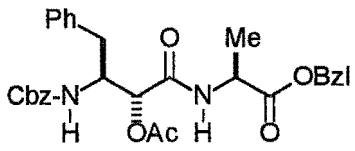
3.39

The acid **3.25** (7 mg, 0.03 mmol) was reacted with *L*-alanine benzyl ester hydrochloride according to general coupling method A. Purification of the crude product by flash chromatography eluting with ethyl acetate-dichloromethane (1:5) gave the amide **3.39** (10 mg, 83%); (Found: M^+ 382.1527. $C_{21}H_{22}N_2O_5$ requires *m/z* 382.1528); ^{1}H NMR, δ 1.50 (3H, d, *J* 7.4, CH_3), 2.42 (1H, dd, *J* 13.2 & 7.7, CHCH_APh), 3.13 (1H, dd, *J* 13.2 & 3.0, CHCH_BPh), 4.42 (1H, m, NC*H*CH_2), 4.70 (1H, m, *C*HMe), 5.10 (1H, d, *J* 8.3, CHO), 5.21 (2H, m, BzlCH_2), 7.17 (2H, m, ArH), 7.26-7.41 (8H, m, ArH).

Benzyl (*2S*)-2-[(*2S,3S*)- and (*2S*)-2-[(*2R,3S*)-2-acetoxy-3-(*N*-benzyloxycarbonylamino)-4-phenyl-butanoylamino]propanoate 3.41 and 3.43



3.41



3.43

The acetate **3.22** or **3.24** (1 equiv; prepared from 3.21 and 3.23, respectively, as described above) was reacted with *L*-alanine benzyl ester hydrochloride (1.1 equiv) in dichloromethane according to general coupling method B. Purification by flash chromatography eluting with ethyl acetate-petroleum ether (3:2) gave the corresponding amide **3.41** or **3.43**. Amide **3.41** (218 mg, 44%); [Found: $(M\text{-}PhCH_2)^+$ 441.1660. $C_{23}H_{25}N_2O_7$ requires *m/z* 441.1661]; $[\alpha]_D^{20}$ -11° (c 0.060 in CH_2Cl_2); ^{1}H NMR, δ 1.41 (3H, d, *J* 7.0, CHCH_3), 2.10 (3H, s, $COCH_3$), 2.90 (1H, dd, *J* 13.7 &

8.8, CHCH_APh), 2.99 (1H, dd, *J* 14.2 & 6.3, CHCH_BPh), 4.39 (1H, m, C*H*CH_2), 4.59 (1H, m, C*H*Me), 5.02 (2H, s, CbzCH_2), 5.14 & 5.20 (2H, ABq, *J* 12.5, BzlCH_2), 5.17 (1H, m, CHOAc), 5.35 (1H, d, *J* 9.5, NHCbz), 6.95 (1H, d, *J* 7.5, N*H*CHMe), 7.22-7.34 (15H, m, ArH); ^{13}C NMR, δ 17.78 (CHCH_3), 20.74 (COCH_3), 36.78 (CHCH_2), 48.11 (*C*HMe), 53.74 (*C*HCH_2), 66.67 (CbzCH_2), 67.25 (BzlCH_2), 74.11 (CHOAc), 126.63, 127.74, 127.97, 128.13, 129.38, 128.46, 128.57 & 129.12 (ArCH), 135.07 & 136.94 (ArC), 155.99 (CbzCO), 167.31, 169.79 & 172.17 (CO); IR /cm^{-1} 1713, 1686, 1506, 1217. Amide **3.43** (501 mg, 51%); [Found: (M-PhCH_2)$^+$ 441.1661. $C_{23}H_{25}N_2O_7$ requires *m/z* 441.1661]; mp 142-144 °C; $[\alpha]_D^{20}$ -19° (c 0.012 in CH_2Cl_2); ^{1}H NMR, δ 1.37 (3H, d, *J* 6.9, CHCH_3), 2.05 (3H, s, COCH_3), 2.84 (2H, d, *J* 7.3, CHCH_2), 4.43 (1H, m, C*H*CH_2), 4.59 (1H, m, C*H*Me), 4.98 & 5.04 (2H, ABq, *J* 12.0, CbzCH_2), 5.14 & 5.21 (2H, ABq, *J* 12.0, BzlCH_2), 5.21 (1H, d, *J* 3.9, CHOAc), 5.47 (1H, d, *J* 9.8, NHCbz), 6.68 (1H, d, J 7.3, N*H*CHMe), 7.17-7.38 (15H, m, ArH); ^{13}C NMR, δ 17.96 (CHCH_3), 20.51 (COCH_3), 37.75 (CHCH_2), 48.01 (*C*HMe), 53.24 (*C*HCH_2), 66.68 (CbzCH_2), 67.28 (BzlCH_2), 73.35 (CHOAc), 126.65, 127.95, 128.36, 128.49, 128.58 & 129.16 (ArCH), 135.07 & 136.89 (ArC), 155.52, (CbzCO), 167.52 & 168.98 (CO).

Preparation of compounds 3.33, 3.35, 3.40, 3.42 and 3.44.

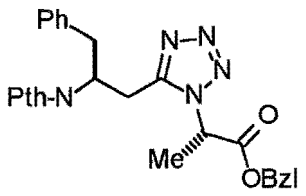
General Method A for Tetrazole Formation (without stereochemical control)

To a stirred suspension of the amide (1 equiv) in dry benzene (5 cm^3/mmol) was added crystalline PCl_5 (1 equiv). A transparent solution formed and the mixture was stirred at rt for 45 min. An extra portion of PCl_5 (0.2 equiv) was added and stirring was continued for a further 45 min. A benzene (3 cm^3) solution of hydrazoic acid (10 equiv) was added and the mixture was stirred at rt for 2 d. The mixture was diluted with benzene (5 cm^3) and washed with aqueous 1M $NaHCO_3$ (3 x 2.5 cm^3), water (2.5 cm^3) and saturated aqueous NaCl (2.5 cm^3). The benzene solution was dried and evaporated to give a mixture of the tetrazole and the unreacted amide which was separated by flash chromatography.

General Method B for Tetrazole Formation (with stereochemical control)

To a stirred solution of PCl_5 (1.2 equiv) in dry chloroform (5 cm^3/mmol) was added quinoline (2.4 equiv) at rt (a white precipitate formed). The mixture was stirred for 30 min before slowly adding a solution of the amide (1 equiv) in chloroform (5 cm^3/mmol) at less than 20 °C. After stirring for 1 h a further portion of PCl_5 (0.2 equiv.) was added and stirring was continued for 2.5 h. A benzene (3 cm^3) solution of hydrazoic acid (30 equiv) was added and the mixture stirred at rt for 2d. The mixture was evaporated, redissolved in ethyl acetate (10 cm^3) and washed with aqueous 2M HCl (2 x 2.5 cm^3), water (2 x 2.5 cm^3) and saturated aqueous NaCl (2.5 cm^3). The solution was dried and evaporated to give a crude mixture of the tetrazole and the unreacted amide which was purified by flash chromatography.

1-[(*1S*)-1-Benzyloxycarbonylethyl]-5-[(*2S*)-3-phenyl-2-(*N*-phthalylamino)propyl]-*1H*-tetrazole 3.33

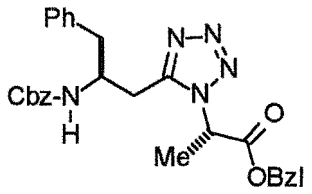


3.33

The amide **3.32** (24 mg, 0.05 mmol) was reacted according to general method A for tetrazole formation. Purification of the crude product (23 mg) by flash chromatography, eluting with ethyl acetate-dichloromethane (3:100 to 3:10), gave two fractions. The first fraction contained **3.33** (13 mg, 51%); (Found: M^+ 495.190. $C_{28}H_{25}N_5O_4$ requires *m/z* 495.1906); R_F 0.59 (1:20 EtOAc-CH_2Cl_2); ^{1}H NMR, δ 1.86 (3H, d, *J* 7.3, CH_3), 3.15-3.31 (3H, m, $CHCH_2Ph$ & CH_2CN_4), 3.74 (1H, dd, *J* 15.6 & 10.3, $CHCH_2Ph$ or CH_2CN_4), 5.08 (1H, m, $CHCH_2$), 5.14 (1H, q, *J* 7.4, CHMe), 5.18 (2H, m, $BzlCH_2$), 7.14-7.37 (10H, m, ArH), 7.65-7.75 (4H, m, PthH); ^{13}C NMR, δ 16.51 (Me), 25.42 (CH_2CN_4), 38.80 ($CHCH_2Ph$), 50.54 ($CHCH_2$), 55.56 (CHMe), 68.32 ($BzlCH_2$), 123.31, 127.04, 128.43, 128.50, 128.67, 128.75, 128.85, 128.98,

131.33, 134.09 & 136.63 (ArC), 152.53 (CN_4), 167.49 & 168.01 (CO); IR /cm^{-1} 1774, 1751, 1712, 1396, 1372. The second fraction contained **3.32** (7 mg, 30%).

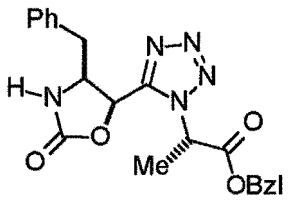
1-[(*1S*)-1-Benzyloxycarbonylethyl]-5-[(*2S*)-2-(*N*-benzyloxycarbonylamino)-3-phenylpropyl]-*1H*-tetrazole 3.35



3.35

The amide **3.34** (978 mg, 2.06 mmol) was reacted according to general method A for tetrazole formation. Purification on a 4 mm chromatatron plate, eluting with ethyl acetate-pentane (3:15 to 2:5), gave two fractions. The first fraction contained **3.35** (484 mg, 47%); (Found: M^+ 499.2223. $C_{28}H_{29}N_5O_4$ requires *m/z* 499.2219); mp 103-104 °C; $[\alpha]_D^{20}$ -51° (c 0.007 in CH_2Cl_2); ^1H NMR, δ 1.88 (3H, d, *J* 7.3, CH_3), 2.87 (1H, dd, *J* 10.2 & 7.5, CHCH_APh), 2.98 (3H, m, CH_2CN_4 & CHCH_BPh), 4.25 (1H, m, C*H*CH_2), 5.01 (1H, q, *J* 7.3, C*H*Me), 5.04 (2H, s, CbzCH_2), 5.08 & 5.14 (2H, ABq, *J* 12.0, BzlCH_2), 5.48 (1H, d, *J* 7.8, NHCbz), 7.08 (2H, m, ArH), 7.19-7.38 (13H, m, ArH); ^{13}C NMR, δ 16.53 (Me), 26.74 (CH_2CN_4), 39.03 ($CHCH_2Ph$), 50.79 ($CHCH_2$), 55.49 (CHMe), 66.75 ($CbzCH_2$), 68.27 ($BzlCH_2$), 126.97, 128.00, 128.18, 128.26, 128.53, 128.73, 128.77, 128.82 & 129.06 (ArCH), 134.32 & 137.10 (ArC), 152.76 (CN4), 157.59 (CbzCO), 167.59 (AlaCO); IR /cm^{-1} 3431, 1751, 1713, 1510. The second fraction contained **3.34** (335 mg, 34%).

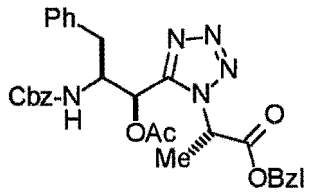
5-[(*4S,5S*)-4-Benzyl-2-oxooxazolidin-5-yl]-1-[(*1S*)-1-benzyloxycarbonylethyl)-*1H*-tetrazole 3.40



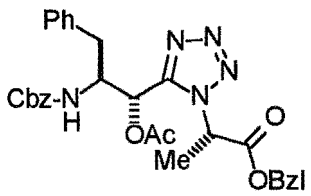
3.40

The amide **3.39** (10 mg, 0.026 mmol) was reacted according to general method B for tetrazole formation to yield a crude mixture of **3.40** and **3.39**. Purification by flash chromatography eluting with ethyl acetate-petroleum ether (1:3 to 1:1) gave a mixture of the tetrazole **3.40** and the amide **3.39** (6 mg, 5:3 by ^{1}H NMR); (Found: M^+ 407.1590. $C_{21}H_{21}N_5O_4$ requires *m/z* 407.1593); ^{1}H NMR, δ **3.40** (from the mixture) 2.01 (3H, d, *J* 7.3, CH_3), 2.36 (1H, m, CHCH_APh), 2.86 (1H, dd, *J* 13.7 & 3.4, CHCH_BPh), 4.49 (1H, m, C*H*CH_2), 5.25 (2H, m, Bz*l*CH_2), 5.70 (1H, q, *J* 7.3, C*H*Me), 6.15 (1H, d, *J* 7.9, CHO), 7.01 (2H, m, ArH), 7.26-7.37 (8H, m, ArH).

5-[(*1S,2S*)- and 5-[(*1R,2S*)-1-Acetoxy-2-(N-benzyloxycarbonylamino)-3-phenylpropyl]-1-[(*1S*)-1-benzyloxycarbonylethyl]-*1H*-tetrazole 3.42 and 3.44



3.42



3.44

The amide **3.41** (184 mg, 0.346 mmol) was reacted according to general method B for tetrazole formation. Purification by flash chromatography, eluting with ethyl acetate-petroleum ether (2:3) gave two fractions. The first fraction contained an oily mixture (17:3 by ^{1}H NMR) of the tetrazoles **3.42** and **3.44** (121 mg, 63% combined yield). Further flash chromatography of this fraction, eluting with ethyl

acetate-petroleum ether (2:3), gave pure **3.42** (25 mg, 13%) and mixtures of **3.42** and **3.44**; **3.42** (Found: M^+ 557.2280. $C_{30}H_{31}N_5O_6$ requires *m/z* 557.2274); $[\alpha]_D^{20}$ -34° (c 0.011 in CH_2Cl_2); ^1H NMR, δ 1.84 (3H, d, *J* 7.4, CHC*H*$_3$), 1.84 (3H, s, $COCH_3$), 2.82 (1H, dd, *J* 14.4 & 7.6, CHC*H*$_A$Ph), 2.93 (1H, dd, *J* 13.9 & 8.6, CHC*H*$_B$Ph), 4.58 (1H, m, C*H*CH_2), 5.02 & 5.08 (2H, ABq, *J* 11.7, $CbzCH_2$), 5.07 & 5.13 (2H, ABq, *J* 12.7, $BzlCH_2$), 5.17 (1H, m, C*H*Me),5.79 (1H, d, *J* 9.3, NH), 6.09 (1H, d, *J* 5.4, CHOAc), 7.01 (2H, m, ArH), 7.21-7.34 (13H, m, ArH); ^{13}C NMR, δ 17.09 (CH*Me*), 20.13 (CO*Me*), 37.06 ($CHCH_2$), 53.83 (*C*HCH_2), 56.28 (*C*HMe), 64.43 (*C*HOAc), 66.81 ($CbzCH_2$), 68.27 ($BzlCH_2$), 127.10, 127.86, 128.13, 128.22, 128.50, 128.67, 128.72 & 128.80 (ArCH), 134.32 & 136.22 (ArC), 151.22 (CN_4), 156.24 (CbzCO), 167.65 & 169.60 (CO); IR /cm^{-1} 1755, 1724, 1512; **3.44** (data given below). The second fraction contained the unreacted amide **3.41** (26 mg, 14%).

The amide **3.43** (455 mg, 0.854 mmol) was also reacted according to general method B for tetrazole formation. Purification by flash chromatography gave two fractions. The first fraction contained a mixture (4:1 by ^1H NMR) of the tetrazoles **3.44** and **3.42** (302 mg, 63%), 15 mg of which was recrystallized from ethyl acetate-petroleum ether to give fine white needles of **3.44** (5 mg); (Found: M^+ 557.2273. $C_{30}H_{31}N_5O_6$ requires *m/z* 557.2274); mp 96-98 °C; $[\alpha]_D^{20}$ -49° (c 0.010 in CH_2Cl_2); ^1H NMR, δ 1.93 (3H, d, *J* 7.3, CHC*H*$_3$), 1.97 (3H, s, $COCH_3$), 2.91 (2H, m, CHC*H*$_2$), 4.42 (1H, m, C*H*CH_2), 5.02 (2H, s, $CbzCH_2$), 5.05 & 5.12 (2H, ABq, *J* 12.2, $BzlCH_2$), 5.36 (1H, m, C*H*Me), 5.92 (1H, d, *J* 5.4, C*H*OAc), 7.07 (2H, m, ArH), 7.20-7.32 (13H, m, ArH); ^{13}C NMR, δ 16.52 (CH*Me*), 19.98 (CO*Me*), 36.38 ($CHCH_2$), 54.38 (*C*HCH_2), 56.12 (*C*HMe), 64.95 (*C*HOAc), 66.68 ($CbzCH_2$), 68.11 ($BzlCH_2$), 126.72, 127.82, 127.97, 128.13, 129.32, 128.49, 128.53, 128.58 & 128.89 (ArCH), 134.23, 136.01 & 136.45 (ArC), 152.00 (CN_4), 155.67 (CbzCO), 167.60 & 169.79 (CO). The second fraction contained the unreacted amide **3.43** (147 mg, 32%).

Preparation of compounds 3.49, 3.50, 3.53, 3.54 and 3.61-3.64

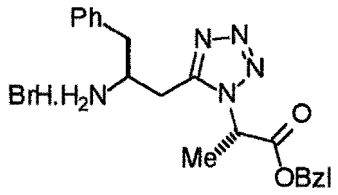
General Method for removal of a Cbz protecting group

To a stirred solution of the protected amine (1 equiv) in acetic acid (1 cm^3) was added 50% HBr in acetic acid (1.9 cm^3/mmol). After 20 min at rt, pre-cooled ether (10 cm^3) was added at -10 °C with vigorous stirring. To the resulting precipitate was added petroleum ether (5 cm^3) and the mixture left to stand at 0 °C for 15 min. The residue was washed with petroleum ether (2 x 5 cm^3) and dried *in vacuo* to give the corresponding amine hydrobromide.

General Method for hydrogenolysis of a benzyl ester

A stirred solution of the benzyl ester (1 equiv) and acetic acid (1 to 3 drops) in ethanol (2.5 to 5 cm^3) was hydrogenated at rt for 18 h in the presence of 10% Pd/C (188 mg/mmol). The mixture was filtered through celite, evaporated, redissolved in sufficient aqueous 1M $NaHCO_3$ and washed with a small amount of ethyl acetate (2 cm^3). The aqueous phase was acidified with solid sodium bisulphite to pH 2.5 and the free acid extracted with ethyl acetate (3 x 10 cm^3), dried and evaporated.

5-[(2S)-2-Amino-3-phenylpropyl]-1-[(*1S*)-1-benzyloxycarbonylethyl]-*1H*-tetrazole hydrobromide 3.45

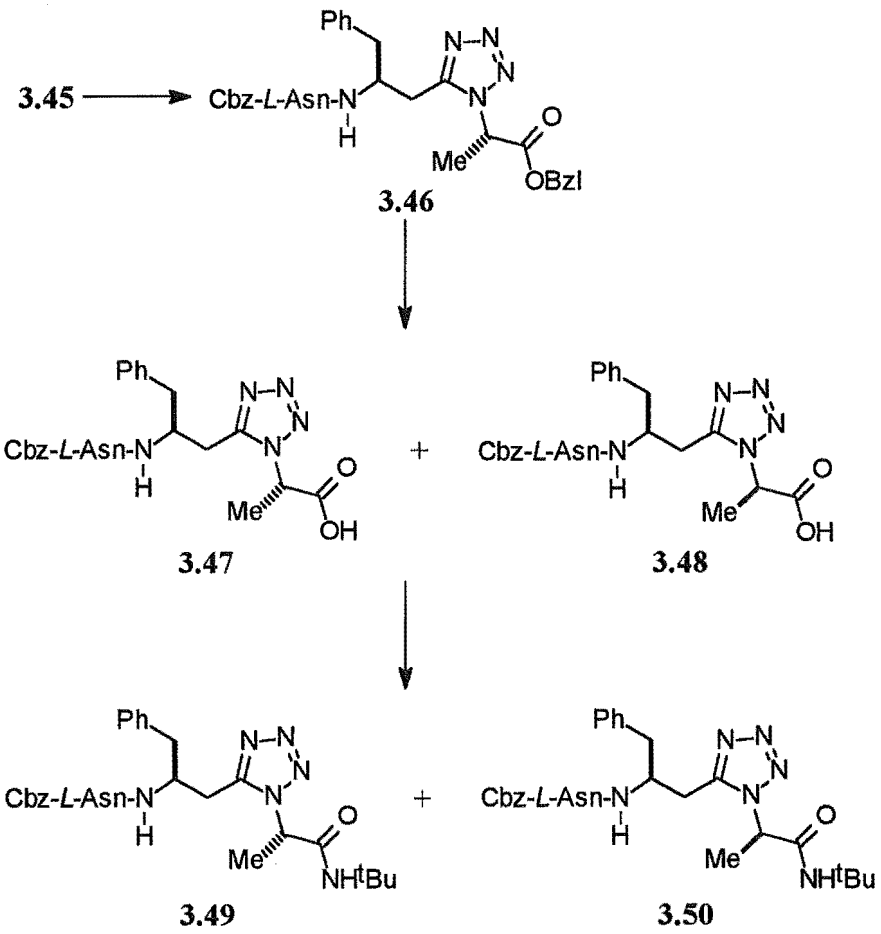


3.45

The tetrazole **3.35** (362 mg, 0.725 mmol) was reacted with 50% HBr in acetic acid, according to the general method, to give the amine hydrobromide **3.45** (271 mg, 84%); (Found: MH^+ 366.1931. $C_{20}H_{24}N_5O_2$ requires *m/z* 366.1929); ^1H NMR

(CD_3OD), δ 1.89 (3H, d, *J* 7.4, $CHCH_3$), 2.99-3.31 (4H, m, $CHCH_2Ph$ & CH_2CN_4), 4.09 (1H, m, $CHCH_2$), 5.14 (2H, s, $BzlCH_2$), 5.61 (1H, q, *J* 7.3, CHMe), 7.26-7.38 (10H, m, ArH).

(*2'S,1''S*) and (*2'S,1''R*)-5-{2-[*N*-(*N*-Benzyloxycarbonyl-*L*-asparaginyl)amino]-3-phenylpropyl}-1-[1-(1,1-dimethylethylaminocarbonyl)ethyl]-*1H*-tetrazole 3.49 and 3.50



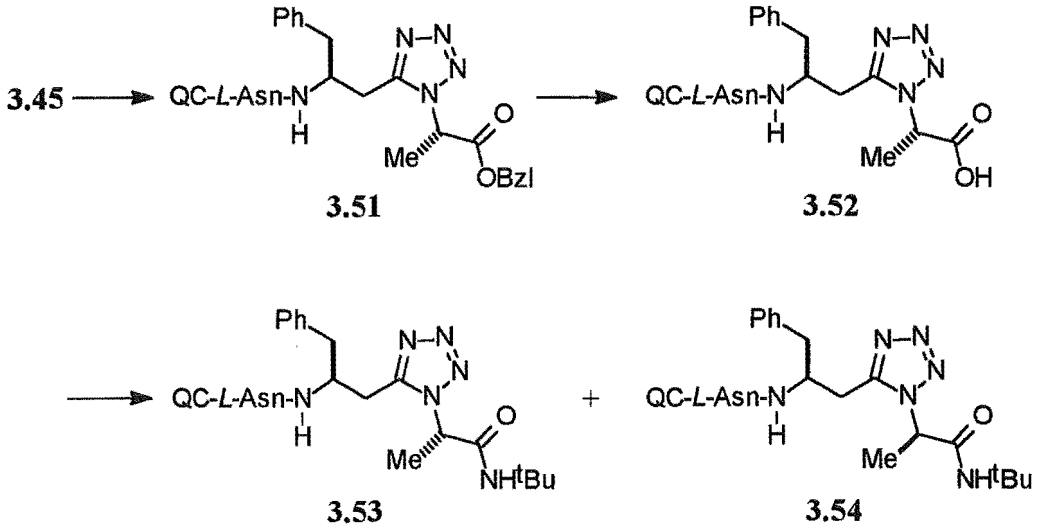
The amine hydrobromide **3.45** (13 mg, 0.03 mmol) was reacted with Cbz-*L*-asparagine according to general coupling method A. Purification by flash chromatography eluting with ethyl acetate-petroleum ether (2:3 to 1:0) gave the amide **3.46** (14 mg, 76%); (Found: MH^+ 614.2730. $C_{32}H_{36}N_7O_6$ requires *m/z* 614.2727); $[\alpha]_D^{20}$ -20° (c 0.010 in CH_2Cl_2); ^1H NMR ($CDCl_3$, d_6-DMSO), δ 1.89 (3H, d, *J* 7.4, $CHCH_3$), 2.38 (2H, m, $AsnCH_2$), 2.79-3.10 (4H, m, $CHCH_2Ph$ & CH_2CN_4), 4.29 (1H, m, AsnCH), 4.40 (1H, m, $CHCH_2Ph$), 5.01 & 5.06 (2H, ABq, *J* 12.2, $BzlCH_2$), 5.16

(2H, s, $CbzCH_2$), 5.59 (1H, q, *J* 6.8, C*H*Me), 6.47 (1H, br s, NH), 7.04 (1H, d, *J* 8.3, AsnNH), 7.16-7.38 (15H, m, ArH), 7.96 (1H, d, *J* 8.3, NH); ^{13}C NMR ($CDCl_3$, d_6-DMSO), δ 14.96 (CH*Me*), 25.47 (*C*H_2CN_4), 36.00 (AsnCH_2), 38.36 (CH*C*H_2Ph), 48.26 (*C*HCH_2Ph), 50.59 (AsnCH), 53.56 (*C*HMe), 64.69 (BzlCH_2), 66.20 ($CbzCH_2$), 124.99, 126.45, 126.59, 126.91, 127.03, 127.10 & 127.77 (ArCH), 136.29 (ArC), 152.20 (CN_4), 169.01, 169.68 & 170.68 (CO).

The benzyl ester **3.46** (13 mg, 0.021 mmol) and potassium hydroxide (1.1 mg, 0.0196 mmol) were dissolved in methanol-water (5 cm^3 of a 4:1 mixture) and stirred at rt for 18 h. The mixture was acidified with aqueous 2M HCl and evaporated to give the acids **3.47** and **3.48** as a mixture of epimers (17:3 by ^1H NMR), which was used without further purification; ^1H NMR ($CDCl_3$, d_6-DMSO), δ **3.47** (from the mixture) 1.88 (3H, d, *J* 7.4, CHC*H*$_3$), 2.46 (2H, m, AsnCH_2), 2.91 (2H, m, CHC*H*$_2$Ph or CH_2CN_4), 3.06 (2H, m, CHC*H*$_2$Ph or CH_2CN_4), 4.33 (1H, m, AsnCH), 4.44 (1H, m, C*H*CH_2Ph), 5.02 (2H, s, $CbzCH_2$), 5.37 (1H, m, C*H*Me), 7.22-7.34 (10H, m, ArH), δ **3.48** (from the mixture) 1.81 (d, *J* 11.3, CHC*H*$_3$). The mixture of acids was reacted with *tert*-butylamine (2 equiv) in dichloromethane according to general coupling method B to give a crude mixture of the amides **3.49** and **3.50**. A mixture of unreacted acids **3.47** and **3.48** (6 mg, 43%) was extracted into the aqueous layer, by the $NaHCO_3$ wash during the workup, and later acidified with solid sodium bisulphite and reextracted with ethyl acetate (3 x 5 cm^3). Purification of the crude products by flash chromatography, eluting with ethyl acetate-methanol (1:0 to 9:1) gave a mixture (3:2 by ^1H NMR) of the amides **3.49** and **3.50** (2 mg, 16%), which were not separated; (Found: MH^+ 579.3040. $C_{29}H_{39}N_8O_5$ requires *m/z* 579.3043); ^1H NMR ($CDCl_3$, d_6-DMSO), δ **3.49** (from the mixture) 1.27 (9H, s, $C(CH_3)_3$), 1.87 (3H, d, *J* 6.8, CHC*H*$_3$), 2.49 (2H, m, AsnCH_2), 2.88-3.06 (4H, m, CHC*H*$_2$Ph & CH_2CN_4), 4.32 (1H, m, AsnCH), 4.37-4.49 (1H, m, C*H*CH_2Ph), 5.03 (2H, m, $CbzCH_2$), 5.21 (1H, q, *J* 7.4, C*H*Me), 6.22 (1H, br s, NH), 6.84 (1H, d, *J* 7.3, NH), 7.18-7.34 (10H, m, ArH), 7.87 (1H, d, *J* 7.8, NH), δ **3.50** (from the mixture) 1.31 (s, $C(CH_3)_3$), 1.78 (d, *J* 6.4, CHC*H*$_3$), 5.14 (q, *J* 6.8, C*H*Me), 7.94 (d, *J* 7.8, NH); ^{13}C NMR ($CDCl_3$, d_6-DMSO), δ 17.52 (CH*Me*), 27.27 (*C*H_2CN_4), 28.03 (*C*Me_3). 36.65 (AsnCH_2), 39.25 (CH*C*H_2Ph), 48.46 (*C*HCH_2Ph), 51.35 (AsnCH), 51.68 (*C*Me_3), 57.32 (*C*HMe), 66.97 ($CbzCH_2$), 126.70, 126.81, 127.82, 128.04, 128.31, 128.47, 128.58, 128.92 & 128.97 (ArCH),

134.45 & 136.74 (ArC); reverse phase HPLC on a C_{18} analytical column [55 % methanol-45 % water(0.1 % TFA)] showed two peaks with retention times of 24:13 min and 25:41 min.

(*1'S,2''S*) and (*1'R,2''S*)-1-[1-(1,1-dimethylethylaminocarbonyl)ethyl]-5-{3-phenyl-2-[*N*-(*N*-quinolinyl-2-carbonyl-*L*-asparaginyl)amino]propyl}-*1H*-tetrazole 3.53 and 3.54



The amine hydrobromide **3.45** (218 mg, 0.49 mmol) was reacted with *N*-(2-quinolinylcarbonyl)-*L*-asparagine[119] (1.1 equiv) in dichloromethane (3 cm^3) and DMF (40 μL) according to the general coupling method B. Purification by flash chromatography, eluting with ethyl acetate-petroleum ether (1:1 to 1:0) gave the amide **3.51** as an oil (186 mg, 60%); (Found: MH^+ 635.2713. $C_{34}H_{35}N_8O_5$ requires *m/z* 635.2730); $[\alpha]_D^{20}$ +12° (c 0.027 in MeOH); ^{1}H NMR, δ 1.88 (3H, d, *J* 7.3, CHC*H*$_3$), 2.71 & 2.83-3.08 (6H, m, AsnCH_2 & CHC*H*$_2$Ph & CH_2CN_4), 4.52 (1H, m, C*H*CH$_2$Ph), 4.96 (1H, m, AsnCH), 5.14 (2H, m, BzlCH_2), 5.17 (1H, q, *J* 7.3, C*H*Me), 5.83 (1H, br s, NH), 6.26 (1H, br s, NH), 7.00-7.11 (5H, m, ArH), 7.23-7.34 (5H, m, ArH), 7.59 (1H, t, QCH), 7.73 (1H, t, QCH), 7.82 (1H, d, QCH), 8.12 (2H, m, QCH), 8.22 (1H, d, QCH), 9.20 (1H, d, *J* 8.3, AsnNH); ^{13}C NMR, δ 16.39 (CH*Me*), 26.98 (*C*H$_2$CN_4), 37.49 (AsnCH_2), 39.87 (CHCH_2Ph), 49.20 (CHCH_2Ph), 50.28 (AsnCH), 55.70 (*C*HMe), 68.32 (BzlCH_2), 118.54, 126.76, 127.57, 128.32, 128.56, 128.68,

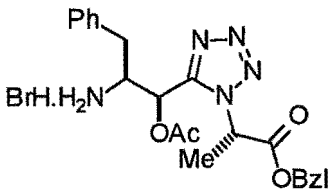
128.74, 129.19, 129.84 & 130.28 (ArCH), 134.50, 136.88 & 137.54 (ArC), 146.34 & 148.21 (QC), 153.27 (CN_4), 165.00, 168.00, 170.78 & 173.61 (CO).

The benzyl ester **3.51** (60 mg, 0.095 mmol) was hydrogenated by the general method to give the acid **3.52** (41 mg, 79%); (Found: MH^+ 545.2265. $C_{27}H_{29}N_8O_5$ requires *m/z* 545.2260); ^{1}H NMR, δ 1.91 (3H, d, *J* 7.3, CHC*H*$_3$), 2.62-2.86 (2H, m, AsnCH_2), 3.10 (4H, m, CH_2Ph & CH_2CN_4), 4.60 (1H, m, C*H*CH_2Ph), 4.92 (1H, m, AsnCH), 5.18 (1H, q, *J* 7.3, C*H*Me), 6.03 (1H, br s, NH), 6.28 (1H, br s, NH), 7.03 (1H, d, *J* 7.4, NH), 7.08-7.23 (5H, m, ArH), 7.65 (1H, t, QCH), 7.80 (1H, t, QCH), 7.88 (1H, d, QCH), 8.17 (2H, m, QCH), 8.32 (1H, d, QCH), 9.17 (1H, d, *J* 8.3, AsnNH); ^{13}C NMR ($CDCl_3$, d_6-DMSO), δ 16.18 (CH*Me*), 26.62 (*C*H_2CN_4), 36.92 (AsnCH_2), 38.96 (CH_2Ph), 49.05 (*C*HCH_2Ph), 49.65 (AsnCH), 55.36 (*C*HMe), 118.28, 126.19, 127.31, 127.77, 128.07, 128.79, 128.87, 129.43 & 129.83 (ArCH), 136.77 & 137.06 (ArC), 146.05 & 148.55 (QC), 152.70 (CN_4), 164.06, 170.07, 170.22 & 172.81 (CO).

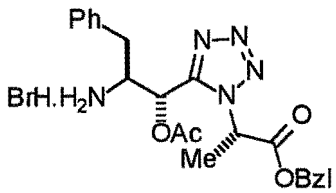
The acid **3.52** (29 mg, 0.053 mmol) was reacted with *tert*-butylamine (1.5 equiv) in dichloromethane according to general coupling method B. Purification by flash chromatography eluting with ethyl acetate-methanol (1:0 to 9:1) gave an epimeric mixture (8 mg, 25%, 1:1 by ^{1}H NMR) of the amides **3.53** and **3.54**. The epimers were separated by reverse phase HPLC on a C_{18} analytical column eluting with 55% methanol-45% water(0.1% TFA). The amide **3.53** eluted first; peak retention time 18:48min; (Found: MNa^+ 622.2875. $C_{31}H_{37}N_9O_4Na$ requires *m/z* 622.2866); $[\alpha]_D^{20}$ +22° (c 0.011 in MeOH); ^{1}H NMR ($CDCl_3$, TFA), δ 1.31 (9H, s, C(CH_3)$_3$), 1.92 (3H, d, *J* 7.3, CHC*H*$_3$), 2.76-3.04 (4H, m, CH_2CN_4 & AsnCH_2), 3.15 (2H, d, *J* 6.4, CH_2Ph), 4.48 (1H, m, C*H*CH_2Ph), 4.92 (1H, m, AsnCH), 5.09 (1H, q, *J* 7.3, C*H*Me), 6.24 (2H, br s, AsnNH_2), 6.44 (1H, br s, NH), 6.97-7.15 (3H, m, ArH), 7.13 (2H, m, ArH), 7.34 (1H, d, *J* 7.8, NH), 7.68 (1H, t, QCH), 7.82 (1H, t, QCH), 7.92 (1H, d, QCH), 8.19 (2H, m, QCH), 8.37 (1H, d, QCH), 9.23 (1H, d, *J* 8.3, AsnNH); ^{13}C NMR ($CDCl_3$, CD_3OD), δ 17.85 (CH*Me*), 27.56 (*C*H_2CN_4), 28.14 (C*Me*$_3$), 39.25 (AsnCH_2 & CH_2Ph), 48.73 (*C*HCH_2Ph), 49.88 (AsnCH), 51.66 (*C*Me$_3$), 57.54 (*C*HMe), 118.53, 126.58, 127.66, 128.25, 128.40, 128.98, 129.62 & 130.30 (ArCH), 136.66 & 137.60 (ArC). The amide **3.54** eluted second; peak retention time 20:40min; (Found: MNa^+ 622.2875. $C_{31}H_{37}N_9O_4Na$ requires *m/z* 622.2866); $[\alpha]_D^{20}$ +14° (c 0.017

in MeOH); ^{1}H NMR ($CDCl_3$, CD_3OD, TFA), δ 1.34 (9H, s, $C(CH_3)_3$), 1.74 (3H, d, *J* 7.3, $CHCH_3$), 2.73 (2H, m, $AsnCH_2$), 2.91-3.13 (4H, m, CH_2CN_4 & CH_2Ph), 4.50 (1H, m, $CHCH_2Ph$), 4.90 (1H, m, AsnCH), 5.02 (1H, q, *J* 6.8, C*H*Me), 7.10-7.20 (5H, m, ArH), 7.33 (1H, d, *J* 7.8, NH), 7.66 (1H, t, QCH), 7.81 (1H, t, QCH), 7.91 (1H, d, QCH), 8.12 (1H, d, QCH), 8.17 (1H, d, QCH), 8.34 (1H, d, QCH); ^{13}C NMR ($CDCl_3$, CD_3OD, TFA), δ 17.56 (CH*Me*), 27.59 (CH_2CN_4), 28.09 (CMe_3), 37.41 ($AsnCH_2$), 38.84 (CH_2Ph), 49.29 ($CHCH_2Ph$), 49.82 (AsnCH), 51.75 (CMe_3), 57.22 (*C*HMe), 118.45, 126.74, 127.60, 128.21, 128.53, 128.89, 129.51 & 130.27 (ArCH), 136.65 & 137.56 (ArC), 146.37 & 148.39 (QC), 165.69 & 166.86 (CO). Unreacted acid was extracted into the aqueous layer by the $NaHCO_3$ wash during the workup. The aqueous washings were acidified with solid sodium bisulphite, extracted with ethyl acetate (3 x 5 cm^3), dried and evaporated to give **3.52** (18 mg, 62%).

(*1'S,2'S,1''S*)- and (*1'R,2'S,1''S*)-5-(1-Acetoxy-2-amino-3-phenylpropyl)-1-(1-benzyloxycarbonylethyl)-*1H*-tetrazole hydrobromide 3.55 and 3.56



3.55

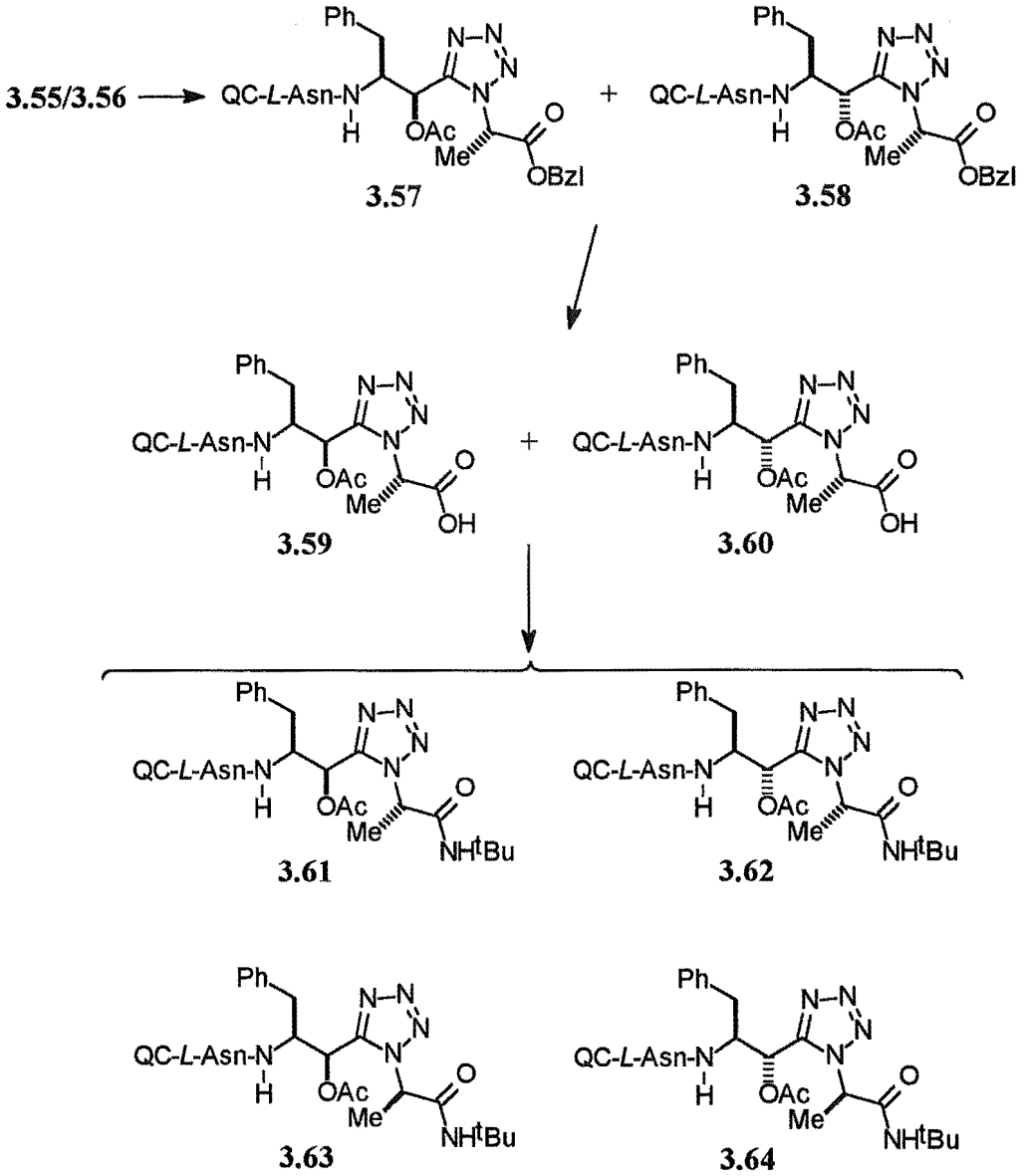


3.56

The tetrazole **3.42** (25 mg, 0.045 mmol) was reacted with 50% HBr in acetic acid, according to the general method, to give the amine hydrobromide **3.55** (17 mg, 84%); ^{1}H NMR (CD_3OD), δ 1.89 (3H, d, *J* 7.4, $CHCH_3$), 2.04 (3H, s, $COCH_3$), 3.03 (2H, m, $CHCH_2$), 4.30 (1H, m, C*H*CH_2), 5.16 (2H, s, $BzlCH_2$), 5.65 (1H, q, *J* 7.3, CHMe), 6.28 (1H, d, *J* 4.4, C*H*OAc), 7.17-7.38 (10H, m, ArH). By an identical procedure, a mixture (3:2 by ^{1}H NMR) of the tetrazoles **3.42** and **3.44** (45 mg, 0.080 mmol) was reacted with 50% HBr in acetic acid to give a mixture (3:2 by ^{1}H NMR) of the amine hydrobromides **3.55** and **3.56** (31 mg, 89%); (Found: MH^+ 424.1991. $C_{22}H_{26}N_5O_4$ requires *m/z* 424.1984); ^{1}H NMR (CD_3OD), δ **3.56** (from the mixture) 1.93 (3H, d, *J* 7.3, $CHCH_3$), 2.16 (3H, s, $COCH_3$), 2.95 (2H, m, $CHCH_2$), 4.08 (1H,

m, C*H*CH_2), 5.07 & 5.13 (2H, ABq, *J* 12.2, BzlCH_2), 5.71 (1H, q, *J* 7.3, C*H*Me), 5.89 (1H, d, *J* 4.4, C*H*OAc), 7.17-7.38 (10H, m, ArH).

(*1'S,2'S,1''S*)-, (*1'R,2'S,1''S*)-, (*1'S,2'S,1''R*)-, and (*1'R,2'S,1''R*)-5-{1-Acetoxy-3-phenyl-2-[*N*-(*N*-quinolinyl-2-carbonyl-*L*-asparaginyl)amino]propyl}-1-[1-(1,1-dimethylethylaminocarbonyl)ethyl]-*1H*-tetrazole 3.61, 3.62, 3.63 and 3.64

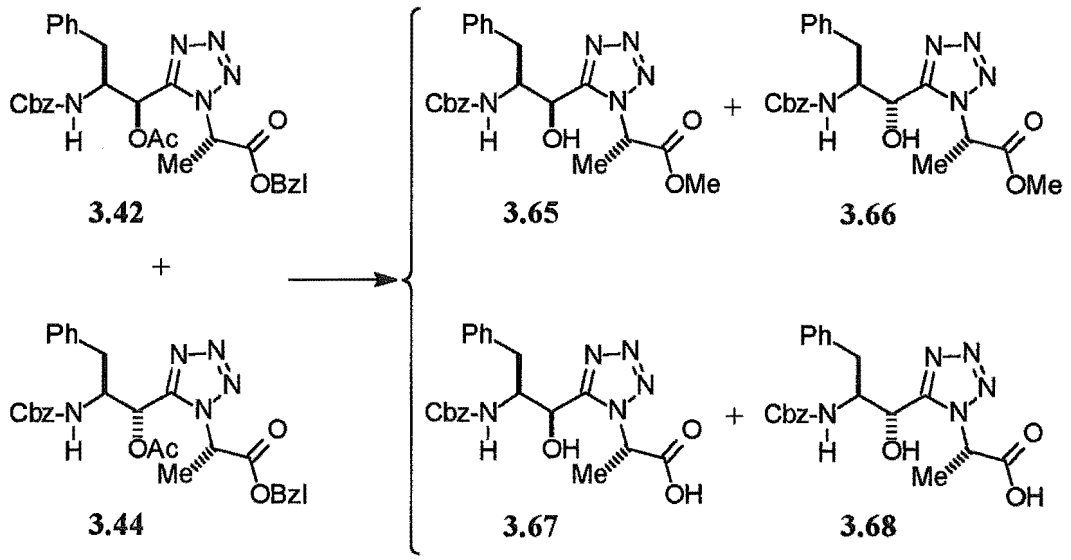


A mixture (31 mg, 0.071 mmol, 3:2 by ^{1}H NMR) of **3.55** and **3.56** was reacted with QC-*L*-Asn (1.3 equiv) and BOP (1.3 equiv) in dichloromethane (5 cm^3)-DMF (0.05 cm^3) for 2 d, according to the general coupling method B. The crude product

(36 mg) contained a mixture of **3.57** and **3.58** (3:2 by ^{1}H NMR) and was used without further purification; ^{1}H NMR, δ **3.57** (from the mixture) 1.93 (3H, s, $COCH_3$), 1.92 (3H, d, *J* 7.3, $CHCH_3$), 2.68-3.18 (4H, m, $CHCH_2Ph$ & $AsnCH_2$), 4.59 (1H, m, $CHCH_2Ph$), 4.86 (1H, m, AsnCH), 5.11 (2H, m, $BzlCH_2$), 5.80 (1H, m, CHMe), 6.36 (1H, d, *J* 5.4, CHOAc), 6.60 (1H, d, *J* 7.8, NH), 7.02-7.36 (10H, m, ArH), 7.57-8.27 (6H, m, QCH), 9.07 (1H, d, *J* 8.3, AsnNH); δ **3.58** (from the mixture) 1.83 (3H, s, $COCH_3$), 1.98 (3H, d, *J* 7.3, $CHCH_3$), 2.68-3.18 (4H, m, $CHCH_2Ph$ & $AsnCH_2$), 4.59 (1H, m, $CHCH_2Ph$), 4.86 (1H, m, AsnCH), 5.11 (2H, m, $BzlCH_2$), 5.80 (1H, m, CHMe), 6.12 (1H, d, *J* 6.3, CHOAc), 6.79 (1H, d, *J* 7.8, NH), 7.02-7.36 (10H, m, ArH), 7.57-8.27 (6H, m, QCH), 9.17 (1H, d, *J* 7.8, AsnNH). The crude mixture of benzyl esters **3.57** and **3.58** (36 mg) was hydrogenated by the general method to give a mixture of the acids **3.59** and **3.60** (15 mg, 3:2 by ^{1}H NMR); ^{1}H NMR, δ 1.84-2.00 (6H, m, $COCH_3$ & $CHCH_3$), 2.66-3.22 (4H, m, CH_2Ph & $AsnCH_2$), 4.70-4.98 ($CHCH_2Ph$ & AsnCH), 5.50, 5.67 (1H, q, *J* 7.3, CHMe), 6.31 (1H, d, *J* 4.4, CHOAc), 6.82-8.30 (11H, m, ArH), 9.12 & 9.23 (1H, d, *J* 8.3, AsnNH). The mixture of acids **3.59** and **3.60** (15 mg, 0.025 mmol) was reacted with *tert*-butylamine (5 equiv), BOP (1.9 equiv) and triethylamine (1.5 equiv) in DMF (0.5 cm^3) according to general coupling method B to give a crude mixture of **3.61**, **3.62**, **3.63** and **3.64** (1:1.5:1.2:1.5 by ^{1}H NMR). Purification by flash chromatography eluting with ethyl acetate-methanol (1:0 to 9:1) gave fractions containing varying mixtures of the four diastereoisomers **3.61-3.64** (4 mg, 24%); (Found: MH^+ 658.3104. $C_{33}H_{40}N_9O_6$ requires *m/z* 658.3101); ^{1}H NMR, δ **3.61** (from the mixtures) 1.29 (9H, s, $C(CH_3)_3$), 2.16 (3H, s, $COCH_3$), 1.97 (3H, d, *J* 7.3, $CHCH_3$), 2.40-2.66, 2.75-2.99, 3.12-3.23 (4H, m, CH_2Ph & $AsnCH_2$), 4.57-4.68 (1H, m, $CHCH_2Ph$), 4.82-4.92 (1H, m, AsnCH), 5.42 (1H, q, *J* 7.3, CHMe), 6.32 (1H, d, *J* 5.4, CHOAc), 7.01-7.38 (5H, m, ArH), 7.65, 7.80, 7.90, 8.08-8.23 & 8.32 (6H, m, QCH), 9.09 (1H, d, *J* 7.3, AsnNH); ^{1}H NMR, δ **3.62** (from the mixtures) 1.33 (9H, s, $C(CH_3)_3$), 2.10 (3H, s, $COCH_3$), 2.06 (3H, d, *J* 7.8, $CHCH_3$), 2.40-2.66, 2.75-2.99, 3.12-3.23 (4H, m, CH_2Ph & $AsnCH_2$), 4.57-4.68 (1H, m, $CHCH_2Ph$), 4.82-4.92 (1H, m, AsnCH), 5.58 (1H, q, *J* 7.3, CHMe), 6.24 (1H, d, *J* 5.4, CHOAc), 7.01-7.38 (5H, m, ArH), 7.65, 7.80, 7.90, 8.08-8.23 & 8.32 (6H, m, QCH), 9.19 (1H, d, *J* 7.8, AsnNH); ^{1}H NMR, δ **3.63** (from the mixtures) 1.35 (9H, s, $C(CH_3)_3$), 2.05 (3H, s, $COCH_3$), 1.82 (3H, d, *J* 6.8,

CHC*H*$_3$), 2.40-2.66, 2.75-2.99, 3.12-3.23 (4H, m, CH_2Ph & $AsnCH_2$), 4.57-4.68 (1H, m, C*H*CH_2Ph), 4.82-4.92 (1H, m, AsnCH), 5.27 (1H, q, *J* 7.3, C*H*Me), 6.22 (1H, d, *J* 4.4, C*H*OAc), 7.01-7.38 (5H, m, ArH), 7.65, 7.80, 7.90, 8.08-8.23 & 8.32 (6H, m, QCH), 9.27 (1H, d, *J* 7.3, AsnNH); ^{1}H NMR, δ **3.64** (from the mixtures) 1.32 (9H, s, $C(CH_3)_3$), 2.13 (3H, s, $COCH_3$), 1.85 (3H, d, *J* 6.9, CHC*H*$_3$), 2.40-2.66, 2.75-2.99, 3.12-3.23 (4H, m, CH_2Ph & $AsnCH_2$), 4.57-4.68 (1H, m, C*H*CH_2Ph), 4.82-4.92 (1H, m, AsnCH), 5.34 (1H, q, *J* 6.8, C*H*Me), 6.18 (1H, d, *J* 5.9, C*H*OAc), 7.01-7.38 (5H, m, ArH), 7.65, 7.80, 7.90, 8.08-8.23 & 8.32 (6H, m, QCH), 8.96 (1H, d, *J* 8.3, AsnNH); reverse phase HPLC on a C_{18} analytical column [55 % methanol-45 % water (0.1 % TFA)] showed four peaks with retention times of 20:46 min, 24:31 min, 28:46 min and 30:46 min. A mixture of the unreacted acids was extracted into the aqueous layer, by the $NaHCO_3$ wash, during the workup. The aqueous washings were acidified with solid sodium bisulphite, extracted with ethyl acetate (3 x 5 cm^3), dried and evaporated to give a mixture of the unreacted acids **3.59** and **3.60** (10 mg, 1:1 by ^{1}H NMR, 67%).

(*1'S,2'S,1''S*)- and (*1'R,2'S,1''S*)-5-[2-(*N*-Benzyloxycarbonylamino)-1-hydroxy-3-phenylpropyl]-1-(1-methoxycarbonylethyl)-*1H*-tetrazole 3.65 and 3.66

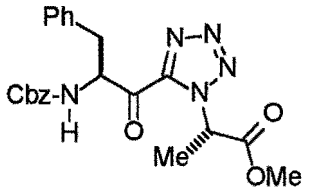


A mixture (24 mg, 0.040 mmol, 4:1 by ^{1}H NMR) of the tetrazoles **3.44** and **3.42** was dissolved in methanol-water (0.4 cm^3 of a 9:1 mixture) with potassium carbonate (1.2 mg, 0.009 mmol) and stirred at rt for 30 min. The mixture was acidified with aqueous 2M HCl and evaporated. Purification by flash chromatography, eluting with ethyl acetate-petroleum ether (3:2), gave two fractions. The first fraction contained a mixture (7:3 by ^{1}H NMR) of the methyl esters **3.66** and **3.65** (7 mg, 40%, see separation and data below). The second fraction contained a mixture (3:1 by ^{1}H NMR) of the free acids **3.68** and **3.67** (5 mg, 29%); ^{1}H NMR (d$_6$-DMSO), δ **3.68** (from the mixture) 1.75 (3H, d, *J* 7.0, CHC*H*$_3$), 2.65-2.85 (2H, m, CHC*H*$_2$), 4.17 (1H, m, C*H*CH$_2$), 4.80 & 4.90 (2H, ABq, *J* 12.5, CbzCH$_2$), 5.03 (1H, d, *J* 4.5, C*H*OH), 5.37 (1H, m, C*H*Me), 7.01 (1H, d, *J* 9.0, NH), 7.12-7.25 (10H, m, ArH), δ **3.67** (from the mixture) 1.66 (3H, d, *J* 7.5, CHC*H*$_3$), 4.98 (1H, d, *J* 6.0, C*H*OH); ^{13}C NMR (d$_6$-DMSO), δ 16.24, 16.35 (CHCH$_3$), 35.14, 36.02 (CHCH$_2$), 55.00, 55.87 (CHMe), 57.70, 58.06 (*C*HCH$_2$), 64.37, 64.43 (CbzCH$_2$), 65.14 (CHOH), 125.10, 125.23, 126.42, 126.47, 126.69, 127.18, 127.30, 127.39, 128.38 & 128.48 (ArCH), 137.88 & 137.95 (ArC), 154.39 & 155.22 (CN$_4$ & CbzCO), 170.96 & 171.35 (CO); *m/z* (FAB), 426 (MH$^+$, 8), 448 (MNa$^+$, 4).

In a similar hydrolysis procedure, a mixture (121 mg, 0.22 mmol, 17:3 by ^{1}H NMR) of the tetrazoles **3.42** and **3.44** was dissolved in methanol-water (5 cm^3 of a 8:2 mixture), with potassium hydroxide (2.2 mg, 0.04 mmol), and stirred at rt for 18 h. The mixture was acidified with aqueous 2M HCl and evaporated. Purification on a 1mm chromatatron plate, eluting with a gradient of petroleum ether-ethyl acetate-methanol (3:2:0 to 0:7:3), gave two fractions. The first fraction contained a mixture (7:3 by ^{1}H NMR) of the methyl esters **3.65** and **3.66** (19 mg, 20%). The second fraction contained a mixture (1:1 by ^{1}H NMR) of the free acids **3.67** and **3.68** (55 mg, 59%).

The methyl esters **3.65** and **3.66** were separated by reverse phase HPLC on a C_{18} analytical column, eluting with 40% methanol-60% water(0.1% TFA). The methyl ester **3.66** eluted first; peak retention time 39:46 min; (Found: M^+ 439.1858. $C_{22}H_{25}N_5O_5$ requires *m/z* 439.1855); ^{1}H NMR, δ 1.88 (3H, d, *J* 7.4, CHC*H*$_3$), 3.15 (2H, m, CHC*H*$_2$), 3.72 (3H, s, OCH_3), 4.29 (1H, m, C*H*CH_2), 4.97 & 5.04 (2H, ABq, *J* 12.4, $CbzCH_2$), 5.10 (1H, d, *J* 3.9, C*H*OH), 5.45 (1H, d, *J* 7.8, NH), 5.69 (1H, q, *J* 7.3, C*H*Me), 7.17-7.31 (10H, m, ArH); ^{13}C NMR, δ 17.05 ($CHCH_3$), 35.98 ($CHCH_2$), 53.16 (OMe), 56.88 (*C*HMe & *C*HCH_2), 67.13 ($CbzCH_2$), 68.04 (CHOH), 126.80, 127.77, 128.17, 128.47, 128.64 & 129.16 (ArCH), 135.83 & 137.30 (ArC), 154.72 (CN_4), 157.31 (CbzCO), 169.19 (CO). The methyl ester **3.65** eluted second; peak retention time 41:31 min; [Found: $(M-CH_4O)^+$ 407.1594. $C_{21}H_{21}N_5O_4$ requires *m/z* 407.1593]; ^{1}H NMR, δ 1.92 (3H, d, *J* 7.4, CHC*H*$_3$), 3.09-3.18 (2H, m, CHC*H*$_2$), 3.70 (3H, s, OCH_3), 4.25 (1H, m, C*H*CH_2), 4.97 & 5.04 (2H, ABq, *J* 12.2, $CbzCH_2$), 5.09 (1H, d, *J* 4.4, C*H*OH), 5.30 (1H, d, *J* 8.3, NH), 5.63 (1H, q, *J* 7.3, C*H*Me), 7.13-7.30 (10H, m, ArH); ^{13}C NMR, δ 16.37 ($CHCH_3$), 36.52 ($CHCH_2$), 53.35 (OMe), 56.62 (*C*HMe & *C*HCH_2), 67.02 ($CbzCH_2$), 67.10 (CHOH), 126.73, 127.71, 128.44, 128.58, 129.04 & 129.14 (ArCH), 135.83 & 136.94 (ArC), 154.08 (CN_4), 157.28 (CbzCO), 169.20 (CO).

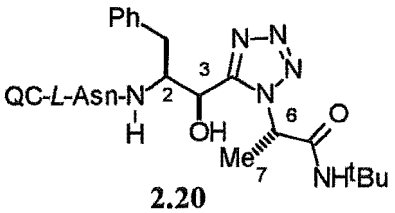
5-[(*2S*)-2-(*N*-Benzyloxycarbonylamino)-3-phenylpropanoyl]-1-[(*1S*)-1-methoxycarbonylethyl]-*1H*-tetrazole 3.70



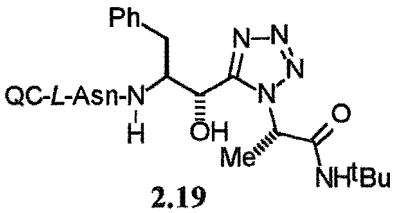
3.70

A mixture of **3.65** and **3.66** (2.7 mg, 0.007 mmol, 1:1 by ^{1}H NMR) and Dess-Martin periodinane[108] (14 mg, 0.035 mmol) was dissolved in dichloromethane (3 cm^3) and stirred at rt for 18 h. To the cloudy solution was added a solution of $Na_2S_2O_3$ (0.049 mmol) in saturated aqueous $NaHCO_3$ (2 cm^3), and the mixture was stirred at rt for 10 min. The mixture was washed with saturated aqueous $NaHCO_3$ (2 cm^3), water (2 cm^3), dried and evaporated to give **3.70** as a colourless oil; (Found: M^+ 437.1701. $C_{22}H_{23}N_5O_5$ requires *m/z* 437.1699); ^{1}H NMR, δ 1.98 (3H, d, *J* 7.3, CHC*H*$_3$), 3.21 (1H, dd, *J* 13.9 & 7.6, CHC*H*$_A$Ph), 3.45 (1H, dd, *J* 14.2 & 4.0, CHC*H*$_B$Ph), 3.75 (3H, s, OMe), 5.04 (2H, s, CbzCH$_2$), 5.33 (1H, d, *J* 6.8, NH), 5.59 (1H, m, C*H*CH$_2$), 5.79 (1H, q, *J* 7.3, C*H*Me), 7.10 (2H, m, ArH), 7.24-7.37 (8H, m, ArH); ^{13}C NMR, δ 16.19 (CH*Me*), 37.39 (CHCH$_2$), 53.39 (OMe), 58.31 (*C*HMe), 59.83 (*C*HCH$_2$), 67.26 (CbzCH$_2$), 127.43, 128.03, 128.29, 128.54, 128.80 & 129.36 (ArCH), 164.79 (CO).

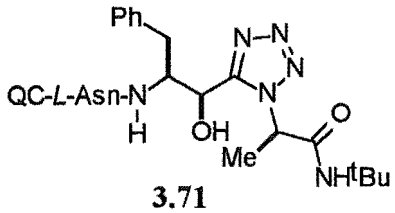
(*1'S,1''S,2''S*)-, (*1'S,1''R,2''S*)-, (*1'R,1''S,2''S*)-, and (*1'R,1''R,2''S*)-1-[1-(1,1-dimethylethylaminocarbonyl)ethyl]-5-{1-hydroxy-3-phenyl-2-[*N*-(*N*-quinolinyl-2-carbonyl-*L*-asparaginyl)amino]propyl}-*1H*-tetrazole 2.20, 2.19, 3.71 and 3.72



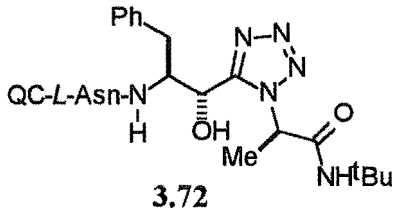
2.20



2.19



3.71



3.72

A mixture of the acetates **3.61-3.64** (4.0 mg, 0.006 mmol) and potassium carbonate (1.7 mg, 0.012 mmol) were dissolved in methanol-water (0.5 cm^3 of a 9:1 mixture), stirred at rt for 18 h and evaporated. The residue was dissolved in ethyl acetate, washed with water, dried and evaporated to give a mixture of the hydroxy compounds **2.20**, **2.19**, **3.71** and **3.72** (3.5 mg, 1:2.8:1.3:2.8 by mass, 95%); (Found: MH^+ 616.2995. $C_{31}H_{38}N_9O_5$ requires *m/z* 616.2995). The four diastereoisomers were separated by reverse phase HPLC on a C_{18} analytical column, eluting with 1:1 methanol-water(0.1 % TFA); peak retention times **2.20** 14:19 min (0.39 mg), **3.71** 16:53 min (0.51 mg), **2.19** 20:11 min (1.11 mg), **3.72** 26:14 min (1.11 mg); ^1H NMR data is shown in table E.1.

Table E.1: ^{1}H NMR (solvent $CDCl_3$, 2 drops CD_3OD) spectroscopic data for **2.19**, **2.20**, **3.71**, **3.72**

Resonance	**2.20** [δ m (*J*)]	**2.19**	**3.71**	**3.72**
$C(Me)_3$	1.30 s	1.31 s	1.29 s	1.32 s
7-H_3	1.93 d (7.3)	1.95 d (7.3)	1.80 d (6.8)	1.79 d (6.9)
AsnCH_2	2.44 & 2.70 dd	2.68 m	2.52 & 2.66 dd	2.75 m
CH_2Ph	2.89 & 3.09 dd	2.83 & 3.15 dd	2.97 & 3.12 dd	2.93 & 3.19 dd
2-H	4.65 m	4.62 m	4.61 m	4.58 m
AsnCH	4.77 m	4.81 m	4.83 m	4.84 m
3-H	5.12 d (5.3)	5.09 d (5.9)	5.04 d (5.9)	5.00 d (5.4)
6-H	5.52 q (6.8)	5.55 q (7.3)	5.42 q (7.3)	5.49 q (6.8)
ArH	7.09-7.15, m	6.87 t 1H, 7.00 t 2H, 7.15 d 2H	7.09-7.13, m	7.01 t 1H, 7.10 t 2H, 7.17 d 2H
QCH	7.67 t, 7.81 t, 7.93 d, 8.17 m 2H, 8.37 d	7.68 t, 7.82 t, 7.94 d, 8.15 m 2H, 8.38 d	7.68 t, 7.81 t, 7.93 d, 8.17 m 2H, 8.38 d	7.67 t, 7.82 t, 7.93 d, 8.14 m 2H, 8.36 d

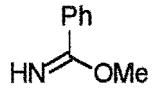
Experimental

Mycalamide Analogues

General procedure

Mps were taken using a Reichert hot-stage microscope and are uncorrected. Optical rotations were measured on a JASCO J-20C recording spectropolarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}, with the concentration given in units of g cm^{-3}. IR spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either a Varian CFT300 or XT300 spectrometer. Samples were run in $CDCl_3$ solution, with Me_4Si as an internal standard, unless otherwise stated. Mass spectra were obtained using a Kratos MS80RFA spectrometer. Radial chromatography was performed on a Chromatatron (Harrison and Harrison) using Merck type 60 PF254 silica gel. Compounds **5.15**, **5.20**, **5.23**, **5.28** and **5.40** were commercially available.

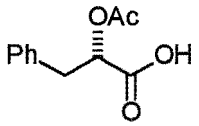
Preparation of methyl benzimidate 5.12



5.12

A solution of benzonitrile (15.0 cm^3, 0.15 mmol) in dry methanol (20.0 cm^3, 0.49 mmol) was saturated with dry hydrogen chloride gas at 4 °C and the solution was stirred for 18 h at 4 °C. Aqueous 5% Na_2CO_3 solution (200 cm^3) was added and the mixture was extracted with dichloromethane (3 x 200 cm^3). The combined organic extracts were dried and evaporated to give[134] **5.12** (21.950 g, quant) as a pale yellow liquid which was not purified further; ^1H NMR ($CDCl_3$), δ 3.92 (3H, s, OMe), 5.26 (1H, s, NH), 7.36-7.44 (5H, m, ArH).

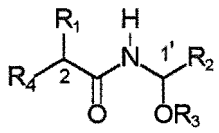
Preparation of (*S*)-2-acetoxy-3-phenylpropanoic acid 5.16



5.16

To a solution of (*S*)-3-phenyllactic acid **5.15** (106 mg, 0.64 mmol) in dry pyridine (1 cm^3) was added acetic anhydride (0.12 cm^3, 1.27mmol) and the mixture was stirred at rt for 18 h. Water (2 cm^3) was added and the solution was extracted with chloroform (3 x 5 cm^3), dried and evaporated to give **5.16** (quant) as a yellow oil which was not purified further. ^{1}H NMR data as previously reported.[149] ^{1}H NMR ($CDCl_3$), δ 2.09 (3H, s, Me), 3.14 (1H, dd, *J* 14.2 & 9.3, CH_APh), 3.28 (1H, dd, *J* 14.2 & 3.9, CH_BPh), 5.27 (1H, dd, *J* 9.3 & 3.9, C*H*O), 7.29 (5H, m, ArH).

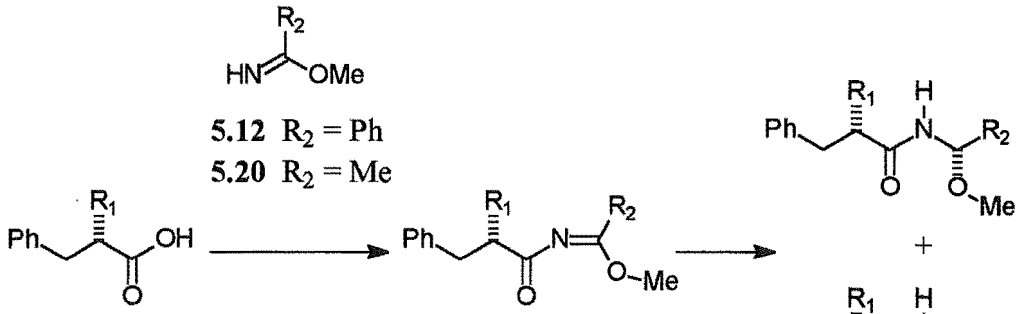
Preparation of compounds based on 5.5



5.5

The general methods A and B detailed below gave essentially pure (1′)-epimeric mixtures of analogues that were unstable to silica. However, for purposes of biological testing, rapid silica-based radial chromatography of the mixtures, where specified, gave samples of the separate isomers in low yield.

General Method A for the preparation of compounds based on 5.5

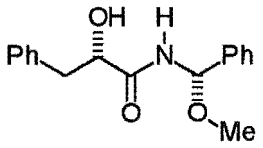


5.12 R_2 = Ph
5.20 R_2 = Me

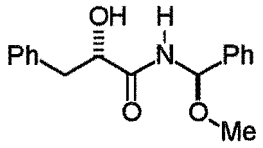
5.16 R_1 = OAc
5.23 R_1 = NH-Cbz
5.28 R_1 = NH-*L*-Ala-Cbz

Compound **5.16**, **5.23** or **5.28** (typically 0.60 mmol), 1-hydroxybenzotriazole (1 equiv) and 1.5 equivalents of **5.12**, (or the hydrochloride salt of **5.20** and 1.5 equivalents of triethylamine) were dissolved in dichloromethane (2.5 cm^3) at 0 °C and the mixture was stirred for 10 min. Dicyclohexylcarbodiimide (DCC, 1 equiv) was added and the mixture stirred for a further 10 min at 0 °C and finally at rt for 18 h. The reaction mixture was diluted with dichloromethane (5 cm^3), filtered and evaporated to give a residue containing the methyl *N*-acylimidate intermediate, which was reacted without further purification. The residue was redissolved in dry isopropyl alcohol (5 cm^3), sodium borohydride (15 equiv) was added and the suspension was stirred at 0 °C for 2 h. Brine (5 cm^3) was added and the mixture extracted with ethyl acetate (3 x 5 cm^3). The combined organic extracts were washed with water (5 cm^3), dried and evaporated to give **5.18/5.19**, **5.21/5.22**, **5.24/5.25** or **5.29/5.30** as mixtures.

(*1'S,2S*)- and (*1'R,2S*)-2-Hydroxy-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.18 and 5.19



5.18



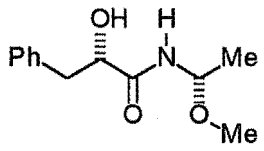
5.19

The acid **5.16**, prepared from **5.15** (96 mg, 0.58 mmol) as previously described, was reacted with **5.12** according to the general method A to give a mixture of **5.18** and **5.19** (54 mg, 33%, 1:1 by ^{1}H NMR). Purification on a 1 mm silica chromatatron plate eluting with diethyl ether-dichloromethane (1:9 to 1:0) gave **5.18** (6 mg). Further elution gave a second fraction (7 mg) containing a mixture of **5.18** and **5.19** (3:2 by ^{1}H NMR). The final fraction gave **5.19** (9 mg) (see below for data).

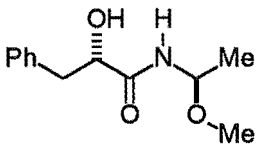
Compounds **5.18** and **5.19** were also prepared by hydrolyzing separate samples of **5.35** (3.7 mg, 0.011 mmol) and **5.36** (4.7 mg, 0.014 mmol) in methanol-water (2.5 cm^3 of a 9:1 mixture) with potassium carbonate (0.2 equiv) at rt for 2 h. The mixtures were evaporated and redissolved in dichloromethane (0.5 cm^3). The organic solutions were washed with water (0.5 cm^3), dried and evaporated to give white crystalline solids **5.18** (3.5 mg) and **5.19** (3.6 mg). **5.18** [Found: (M - Me)$^+$, 270.1125. $C_{16}H_{16}NO_3$ requires *m/z* 270.1130]; mp 104-105 °C; $[\alpha]_D^{23}$ -92 (c 0.038 in CH_2Cl_2); IR /cm^{-1} 3406, 1684, 1504, 1497, 1092; ^{1}H NMR, δ 2.61 (1H, d, *J* 4.9, OH), 2.96 (1H, dd, *J* 14.0 & 8.5, CH_2Ph), 3.27 (1H, dd, *J* 14.0 & 4.2, CH_2Ph), 3.37 (3H, s, OMe), 4.32 (1H, m, C*H*CH_2), 6.10 (1H, d, *J* 9.3, NCH), 7.00 (1H, d, *J* 9.8, NH), 7.30-7.38 (10H, m, ArH); ^{13}C NMR, δ 40.82 (CH_2Ph), 55.88 (OMe), 72.85 (CHOH), 81.16 (NCH), 125.79, 127.10, 128.56, 128.58, 128.78 & 129.55 (ArCH), 136.48 & 139.93 (ArCR), 172.82 (CONH); *m/z* (EI) 270 (M$^+$ - Me, 15%), 253 (M$^+$ - MeOH, 22), 121 (99), 106 (100). **5.19** [Found: (M - Me)$^+$, 270.1128. $C_{16}H_{16}NO_3$ requires *m/z* 270.1130]; mp 66-68 °C; $[\alpha]_D^{23}$ -17 (c 0.036 in CH_2Cl_2); IR /cm^{-1} 3406, 1684, 1504, 1497, 1090; ^{1}H NMR, δ 2.96 (1H, dd, *J* 13.8 & 8.1, CH_2Ph), 3.27 (1H, dd, *J* 13.9 & 4.1, CH_2Ph), 3.47 (3H, s, OMe), 4.45 (1H, dd, *J* 8.3 & 3.9, C*H*CH_2), 6.12 (1H, d, *J* 9.3, NCH), 7.01 (1H, d, *J* 9.3, NH), 7.31 (10H, m, ArH); ^{13}C NMR, δ 40.59 (CH_2Ph),

56.09 (OMe), 72.66 (CHOH), 81.07 (NCH), 125.82, 127.09, 128.49, 128.54, 128.78 & 129.61 (ArCH), 136.34 & 138.76 (ArCR), 172.85 (CONH); *m/z* (EI) 270 (M^+ - Me, 7), 253 (M^+ - MeOH, 17), 121 (77), 106 (100).

(*1'S,2S*)- and (*1'R,2S*)-2-Hydroxy-*N*-(1-methoxyethyl)-3-phenylpropanamide 5.21 and 5.22



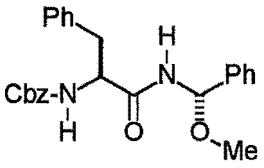
5.21



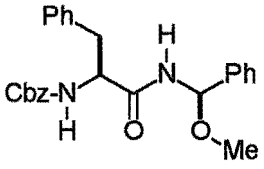
5.22

The acid **5.16**, prepared from **5.15** (100 mg, 0.60 mmol) as previously described, was reacted with **5.20** according to the general method A to give a mixture of **5.21** and **5.22** (55 mg, 42%, 1:1 by ^{1}H NMR). Purification on a preparative silica column eluting with methanol-water (92:5) gave three fractions. The first fraction (11 mg) contained a mixture of **5.22** and **5.21** (1:1 by ^{1}H NMR). The second fraction (6 mg) contained a mixture of **5.22** and **5.21** (3:2 by ^{1}H NMR); ^{1}H NMR, δ **5.21** (from the mixture) 1.30 (3H, d, *J* 5.8, CH*Me*), 2.94 (1H, m, CH_2Ph), 3.28 (1H, m, CH_2Ph), 3.24 (3H, s, OMe), 4.36 (1H, m, C*H*CH_2), 5.26 (1H, m, NCH), 7.00 (1H, d, NH), 7.28 (5H, m, ArH). The final fraction gave **5.22** as an oil (1 mg); [Found: $(M - Me)^+$, 208.0966. $C_{11}H_{14}NO_3$ requires *m/z* 208.0973]; ^{1}H NMR, δ 1.26 (3H, d, *J* 5.8, CH*Me*), 2.90 (1H, dd, *J* 9.6 & 8.3, CH_2Ph), 3.24 (1H, m, CH_2Ph), 3.30 (3H, s, OMe), 4.41 (1H, dd, *J* 8.3 & 3.4, C*H*CH_2), 5.25 (1H, dd, *J* 9.7 & 5.8, NCH), 7.03 (1H, d, *J* 9.8, NH), 7.25 (5H, m, ArH); *m/z* (EI) 208 (M^+ - Me, 2), 205 (M^+ - H_2O, 6), 191 (M^+ - MeOH, 80), 91 (100).

(*1'S,2S*)- and (*1'R,2S*)-2-(*N*-Benzyloxycarbonylamino)-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.24 and 5.25



5.24

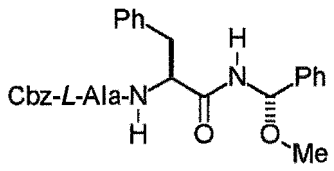


5.25

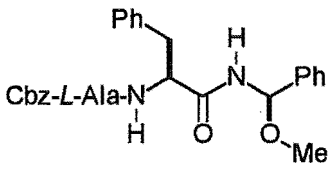
The acid **5.23** (203 mg, 0.68 mmol) was reacted with **5.12** according to the general method A to give a crude mixture of **5.24** and **5.25**. Purification on a 2 mm silica chromatatron plate eluting with ethyl acetate gave two fractions. The first fraction (118 mg) contained a mixture of **5.24**, **5.25** and dicyclohexylurea (1:1:1 by ^{1}H NMR). The second fraction gave *N*-Cbz-*L*-phenylalaninamide[150] **5.26** (73 mg, 37%) as a white solid which was used to prepare **5.51** and **5.52** by general method B; ^{1}H NMR, δ **5.26** 3.10 (2H, m, CHC*H*$_2$), 4.45 (1H, m, C*H*CH$_2$), 5.08 (2H, s, CbzCH$_2$), 5.45 (1H, br d, NHCbz), 5.53 (1H, br s, NH), 5.88 (1H, br s, NH), 7.20-7.35 (10H, m, ArH). Fraction one was further purified on a 1 mm chromatatron plate eluting with ethyl acetate-pentane (1:20 to 1:3) to give two fractions. The first fraction (37 mg) contained a mixture of **5.24** and **5.25** (7:3 by ^{1}H NMR); [Found: (M - Me)$^+$, 403.1667. $C_{24}H_{23}N_2O_4$ requires *m/z* 403.1658]; mp 151-157 °C; ^{1}H NMR (CDCl$_3$, d$_5$-pyridine), δ **5.24** (from the mixture) 3.15 (2H, d, *J* 6.8, CHC*H*$_2$), 3.32 (3H, s, OMe), 4.60 (1H, m, C*H*CH$_2$), 5.04 (2H, s, CbzCH$_2$), 6.09 (1H, d, *J* 9.3, NCHO), 7.14-7.31 (15H, m, ArH); ^{13}C NMR (CDCl$_3$, d$_5$-pyridine), δ **5.24** (from the mixture) 38.33 (CHCH$_2$), 55.67 (OMe), 56.21 (CHCH$_2$), 66.70 (CbzCH$_2$), 81.37 (NCHO), 125.68, 126.73, 127.74, 127.90, 128.11, 128.18, 128.26, 128.38 & 129.19 (ArCH), 136.09 & 138.76 (ArCR), 155.85 (CbzCO), 171.48 (CONH); *m/z* (EI) 403 (M$^+$ - Me, 32), 386 (M$^+$ - MeOH, 100). The second fraction (22 mg) was rechromatagraphed on a 1 mm chromatatron plate eluting with ethyl acetate-petroleum ether (1:4) to give three further fractions. The first fraction (2 mg) contained a mixture of **5.24** and **5.25** (1:1 by ^{1}H NMR). The second fraction (3 mg) contained a mixture of **5.25** and **5.24** (17:3 by ^{1}H NMR). The final fraction (2 mg) contained **5.25**; (Found: M$^+$, 418.1884.

$C_{25}H_{26}N_2O_4$ requires *m/z* 418.1893); mp 151-153 °C; ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ 3.08 (2H, d, *J* 6.8, CHC*H*$_2$), 3.40 (3H, s, OMe), 4.53 (1H, m, C*H*CH$_2$), 5.07 (2H, s, CbzCH$_2$), 6.07 (1H, d, *J* 9.7, NCHO), 7.10-7.31 (15H, m, ArH), 7.45 (1H, d, *J* 9.3, NH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 38.56 (CHCH$_2$), 55.79 (OMe), 56.44 (CHCH$_2$), 66.81 (CbzCH$_2$), 81.29 (NCHO), 125.73, 126.77, 127.82, 127.99, 128.14, 128.25, 128.36, 128.49, 129.20 & 129.27 (ArCH), 136.15 & 138.73 (ArCR), 155.85 (CbzCO), 171.37 (CONH); *m/z* (EI) 418 (M$^+$, 5), 403 (M$^+$ - Me), 386 (M$^+$ - MeOH, 100).

(*1'S,2S*)- and (*1'R,2S*)-2-[*N*-(*N*-Benzyloxycarbonyl-*L*-alaninyl)amino]-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.29 and 5.30



5.29

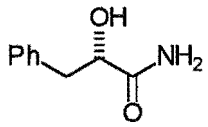


5.30

The acid **5.28** (50 mg, 0.13 mmol) was reacted with **5.12** according to the general method A to give a mixture of **5.29** and **5.30** (36 mg, 54%, 1:1 by ^{1}H NMR) which was loaded onto a 1 mm chromatatron plate and eluted with ethyl acetate-petroleum ether (1:3 to 3:1) to give three fractions. The first fraction (9 mg) contained **5.30**; [Found: (M - MeOH)$^+$, 457.1993. $C_{27}H_{27}N_3O_4$ requires *m/z* 457.2001]; ^{1}H NMR, δ 1.20 (3H, d, *J* 6.8, CH*Me*), 3.11 (2H, d, *J* 6.8, CHC*H*$_2$), 3.30 (3H, s, OMe), 4.24 (1H, m, C*H*Me), 4.76 (1H, m, C*H*CH$_2$), 4.92 (2H, m, CbzCH$_2$), 5.50 (1H, d, *J* 6.3, AlaNH), 6.02 (1H, d, *J* 9.3, NCHO), 7.06-7.30 (15H, m, ArH); ^{13}C NMR, δ 18.07 (CH*Me*), 37.71 (CHCH$_2$), 50.96 (*C*HMe), 54.25 (*C*HCH$_2$), 55.83 (OMe), 67.11 (CbzCH$_2$), 81.61 (NCHO), 125.85, 125.91, 126.91, 126.99, 128.07, 128.31, 128.49, 128.61 & 129.26 (ArCH), 136.08 & 138.54 (ArCR), 171.05 & 172.28 (CO); m/z (EI) 457 (M$^+$ - MeOH, 2), 352 (4), 91 (100). The second fraction (11 mg) gave a mixture of **5.29** and **5.30** (1:1 by ^{1}H NMR); for comparison with the **5.30** data above, ^{1}H NMR, δ **5.29** (from the mixture) 1.24 (3H, d, *J* 7.3, CH*Me*), 3.04 (2H, d, *J* 3.6,

CHC*H*$_2$), 3.31 (3H, s, OMe), 4.36 (1H, m, C*H*Me), 4.83 (1H, m, C*H*CH$_2$), 5.00 (2H, m, CbzCH$_2$), 5.73 (1H, d, *J* 7.3, AlaNH), 5.96 (1H, d, *J* 9.3, NCHO), 7.06-7.30 (15H, m, ArH). Crystallisation of the second fraction from $CDCl_3$ gave **5.29**; [Found: (M - MeOH)$^+$, 457.2005. $C_{27}H_{27}N_3O_4$ requires *m/z* 457.2002]; ^{1}H NMR ($CDCl_3$, d_6-DMSO), δ 1.23 (3H, d, *J* 7.3, CH*Me*), 3.01 (1H, m, CHC*H*$_2$), 3.15 (1H, m, CHC*H*$_2$), 3.39 (3H, s, OMe), 4.12 (1H, m, C*H*Me), 4.71 (1H, m, C*H*CH$_2$), 5.05 (2H, m, CbzCH$_2$), 6.04 (1H, d, *J* 9.3, NCHO), 6.86 (1H, d, *J* 7.3, AlaNH), 7.17-7.34 (15H, m, ArH), 7.72 (1H, d, *J* 9.3, PheNH), 8.23 (1H, d, *J* 9.3, CONH); ^{13}C NMR ($CDCl_3$, d_6-DMSO), δ 17.16 (CH*Me*), 36.63 (CHCH$_2$), 49.87 (*C*HMe), 53.32 (*C*HCH$_2$), 54.47 (OMe), 65.30 (CbzCH$_2$), 80.17 (NCHO), 125.08, 125.42, 127.01, 127.14, 127.35 & 128.36 (ArCH), 136.06 & 138.19 (ArCR), 170.60, 171.58 (CO). The final fraction gave more **5.29** (10 mg).

Preparation[136] of (*S*)-3-phenyllactamide 5.27

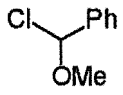


5.27

Concentrated sulphuric acid (52 μL, 0.98 mmol) was added with stirring to (*S*)-3-phenyllactic acid **5.15** (155 mg, 0.94 mmol), dissolved in dry acetone (0.7 cm^3) at -10 °C. An ice cold solution of sodium carbonate (200 mg, 1.88 mmol) in water (2.2 cm^3) was added slowly and the reaction mixture evaporated to dryness under reduced pressure. Liquid ammonia (5 cm^3) was added to the residue and the solution was stirred at rt for 18 h. The mixture was evaporated and partitioned between chloroform and water. The aqueous layer was extracted with chloroform (3 x 5 cm^3) and ethyl acetate (3 x 5 cm^3). The combined organic extracts were dried and evaporated to give **5.27** as a white solid (97 mg, 63%), (Found: MH$^+$, 166.0867. $C_9H_{12}NO_2$ requires *m/z* 166.0868); mp 111-112 °C (literature[151] mp 109-112 °C); $[\alpha]_D^{23}$ -71 (*c* 0.050 in CH_2Cl_2); ^{1}H NMR, δ 2.74 (1H, d, *J* 3.9, OH), 2.91 (1H, dd, *J* 13.7 & 8.8, C*H*$_2$), 3.24 (1H, dd, *J* 14.0 & 4.2, C*H*$_2$), 4.32 (1H, m, C*H*), 5.64 (1H, br s,

NH), 6.44 (1H, br s, NH), 7.25-7.37 (5H, m, ArH); ^{13}C NMR, δ 40.78 (CH_2), 72.77 (CH), 127.10, 128.81 & 129.51 (ArCH), 136.71 (ArCR), 175.47 (CO); *m/z* (EI) 166 (MH^+, 1), 147 ($M^+ - H_2O$, 82), 91 (100).

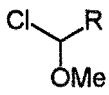
Preparation[137] of methyl 1–chlorobenzyl ether 5.34



5.34

A mixture of freshly distilled benzaldehyde (5.0 cm^3, 49.30 mmol), dry methanol (2.0 cm^3, 49.30 mmol) and anhydrous sodium sulphate (4.8 g) in ethyl chloride (6 cm^3), at -60 °C, was saturated with dry gaseous hydrogen chloride and left to stand at that temperature for 1 h. The excess hydrogen chloride gas was removed by flushing the solution with dry nitrogen gas for 15 min and the mixture was stored at -10 °C over anhydrous $CaSO_4$. Analysis by ^{1}H NMR, by comparing the integrals of the aldehyde CHO resonance with the characteristic new CHCl resonance, indicated that the solution contained a mixture of the α-chloro ether **5.34** and benzaldehyde (17:3), with some ethyl chloride present; ^{1}H NMR, δ 3.69 (3H, s, OMe), 6.47 (1H, s, CHCl), 7.32-7.41 (3H, m, ArH), 7.50 (2H, m, ArH).

General Method[137] for the preparation of the α-chloro ethers 5.37-5.39



5.37 R = Et
5.38 R = Me
5.39 R = *i*Pr

Dry gaseous hydrogen chloride was bubbled through a solution of the aldehyde (propanal, ethanal or 2-methylpropanal, typically 0.179 mol) and dry methanol (7.25 cm^3, 0.179 mol) at -30 °C for 1 h. The excess HCl gas was removed by flushing the solution with dry nitrogen gas for 15 min to give mixtures of **5.37-5.39** and the corresponding aldehydes, which could be stored at -10 °C over anhydrous $CaSO_4$.

The products were analyzed by ^{1}H NMR, by comparing the integrals of the aldehyde CHO resonance with the characteristic new CHCl resonance of the α-chloro ether.

1-chloropropyl methyl ether 5.37

Reaction with propanal gave a mixture of **5.37** and propanal (17:3 by ^{1}H NMR); ^{1}H NMR, δ **5.37** (from the mixture) 1.04 (3H, t, *J* 7.6, CH_2CH_3), 2.02 (2H, m, CH_2CH_3), 3.53 (3H, s, OMe), 5,46 (1H, t, *J* 5.4, CHCl).

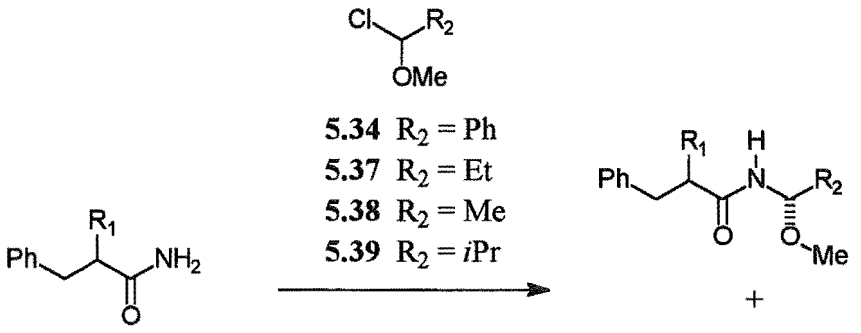
1-chloroethyl methyl ether 5.38.

Reaction with ethanal gave a mixture of **5.38** and ethanal (17:3 by ^{1}H NMR); ^{1}H NMR data as previously reported[152]; ^{1}H NMR, δ **5.38** (from the mixture) 1.78 (3H, d, *J* 5.4, $CHCH_3$), 3.51 (3H, s, OMe), 5.63 (1H, q, *J* 5.3, CHCl).

1-chloro-2-methylpropyl methyl ether 5.39

Reaction with 2-methylpropanal gave a mixture of **5.39** and 2-methylpropanal (9:1 by ^{1}H NMR); ^{1}H NMR, δ **5.39** (from the mixture) 1.02 (3H, d, *J* 1.4 Hz, $CHCH_3$), 1.05 (3H, d, *J* 1.5 Hz, $CHCH_3$), 2.14 (1H, m, $CHMe_2$), 3.52 (3H, s, OMe), 5.36 (1H, d, *J* 3.9 Hz, CHCl).

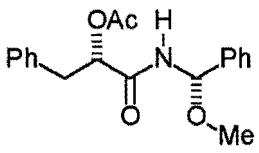
General Method B for the preparation of analogues based on 5.5



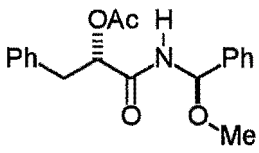
5.33 R_1 = (*S*)-OAc
5.42 R_1 = (*R*)-OAc
5.26 R_1 = (*S*)-NH-Cbz
5.59 R_1 = (*S*)-OCOPhBr
5.62 R_1 = (*S*)-Ocamphanyl

Triethylamine (25 equiv) and either **5.34**, **5.37**, **5.38** or **5.39** (25 equiv, see preparation above) were added to **5.33**, **5.42**, **5.26**, **5.59** or **5.62** (typically 0.3 mmol), dissolved in dry dichloromethane (2.5 cm^3) at 0 °C. The mixture was allowed to warm to rt and then stirring was continued at 5 °C for 18 h. The reaction mixture was washed with water, dried and evaporated to give **5.35/5.36**, **5.43/5.44**, **5.47/5.48**, **5.51/5.52**, **5.60/5.61** or **5.63/5.64** as (1′)-epimeric mixtures.

(*1′S,2S*)- and (*1′R,2S*)-2-Acetoxy-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.35 and 5.36



5.35

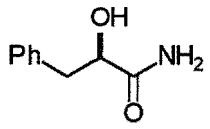


5.36

The amide **5.27** (87 mg, 0.53 mmol) was acetylated with acetic anhydride (0.15 cm^3, 1.59 mmol) in pyridine (3 cm^3) to give **5.33**[153] as a yellow oil (quant), which was not purified further; ^1H NMR, δ 2.07 (3H, s, Ac), 3.19 (2H, m, CH_2), 5.39 (1H, m,

CH), 6.09 (1H, br s, NH), 6.17 (1H, br s, NH), 7.18-7.35 (5H, m, ArH). The amide **5.33** (0.53 mmol) was reacted with **5.34** according to the general method B to give a crude mixture (296 mg) of **5.35** and **5.36** and benzaldehyde. Purification on a 2 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:1) gave a mixture of **5.35** and **5.36** (144 mg, 83%, 1:1 by ^{1}H NMR). Further purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:20 to 1:1) gave three fractions. The first fraction (21 mg) contained a mixture of **5.36** and **5.35** (9:1 by ^{1}H NMR). Recrystallization from ethyl acetate-petroleum ether gave **5.36** (6 mg); [Found: $(M - Me)^+$, 312.1233. $C_{18}H_{18}NO_4$ requires *m/z* 312.1236]; mp 122-125 °C; $[\alpha]_D^{23}$ +89 (c 0.053 in CH_2Cl_2); IR /cm^{-1} 3419, 1747, 1693, 1506, 1454, 1373, 1220; ^{1}H NMR, δ 2.07 (3H, s, Ac), 3.21 (2H, dd, *J* 5.6 & 2.2, CH_2Ph), 3.41 (3H, s, OMe), 5.49 (1H, m, C*H*CH_2), 6.11 (1H, d, *J* 9.8, NCH), 6.46 (1H, d, *J* 9.7, NH), 7.12-7.30 (10H, m, ArH); ^{13}C NMR, δ 20.85 (Ac), 37.49 (CH_2Ph), 56.12 (OMe) 74.17 (*C*HCH_2), 80.82 (NCH), 125.71, 126.94, 128.31, 128.35, 128.40 & 129.66 (ArCH), 135.87 & 138.57 (ArCR), 149.71 (AcCO), 169.32 (CONH); *m/z* (EI) 312 (M^+ - Me, 8), 296 (M^+ - OMe, 15), 237 (50), 106 (100). The second fraction (56 mg) contained a mixture of **5.35** and **5.36** (7:3 by ^{1}H NMR). The final fraction (28 mg) contained a mixture of **5.35** and **5.36** (9:1 by ^{1}H NMR). Recrystallization from ethyl acetate-petroleum ether gave **5.35** (5 mg); [Found: $(M - Me)^+$, 312.1234. $C_{18}H_{18}NO_4$ requires *m/z* 312.1236]; mp 110-112 °C; $[\alpha]_D^{23}$ -25 (c 0.053 in CH_2Cl_2); IR /cm^{-1} 3419, 1747, 1693, 1506, 1454, 1373, 1220; ^{1}H NMR, δ 2.04 (3H, s, Ac), 3.22 (2H, m, CH_2Ph), 3.33 (3H, s, OMe), 5.36 (1H, m, C*H*CH_2), 6.10 (1H, d, *J* 9.2, NCH), 6.77 (1H, d, NH), 7.17-7.33 (10H, m, ArH); ^{13}C NMR, δ 20.72 (Ac), 37.48 (CH_2Ph), 55.88 (OMe), 74.50 (*C*HCH_2), 80.97 (NCH), 123.62, 125.61, 126.91, 128.28, 128.36, 129.54 (ArCH), 135.86, 138.87 (ArCR), 149.64 (AcCO), 169.32 (CONH); *m/z* (EI) 312 (M^+ - Me, 1%), 296 (M^+ - OMe, 8), 252 (84), 121 (100).

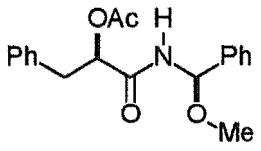
Preparation of (*R*)-3-phenyllactamide 5.41



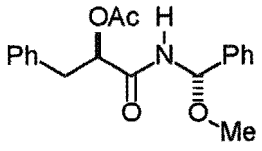
5.41

Prepared from (*R*)-3-phenyllactic acid **5.40** (174 mg, 1.04 mmol), as for **5.27**, to give **5.41** as fine white crystals (133 mg, 77%); mp 111-112 °C (literature[151] mp 109-112 °C); $[\alpha]_D^{23}$ +63 (c 0.050 in CH_2Cl_2); NMR data identical to enantiomer **5.27**.

(*1'R,2R*)- and (*1'S,2R*)-2-Acetoxy-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.43 and 5.44



5.43

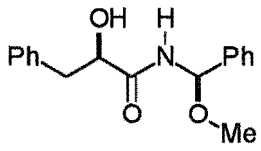


5.44

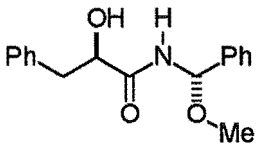
The amide **5.41** (40 mg, 0.24 mmol) was acetylated with acetic anhydride (69 μL, 0.73 mmol) in pyridine (3 cm^3) to give **5.42** as a yellow oil (quant), which was not purified further; ^1H NMR identical to enantiomer **5.33**. The amide **5.42** (0.24 mmol) was reacted with **5.34** according to the general method B to give a mixture (33 mg, 41%) of **5.43** and **5.44** (1:1 by ^1H NMR). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (2:25 to 1:3) gave four fractions. The first fraction (2 mg) contained a mixture of **5.44** and **5.43** (9:1 by ^1H NMR). The second fraction (5 mg) contained a mixture of **5.44** and **5.43** (7:3 by ^1H NMR). Recrystallization from ethyl acetate-petroleum ether gave **5.44** (3 mg); [Found: (M^+ - Me), 312.1233. $C_{18}H_{18}NO_4$ requires *m/z* 312.1236]; mp 129-131 °C; $[\alpha]_D^{23}$ -37 (c 0.023 in CH_2Cl_2); NMR data identical to enantiomer **5.36**; *m/z* (EI) 312 (M^+ - Me, 0.2), 296 (M^+ - OMe, 2), 252 (47), 121 (100). The third fraction (5 mg) contained a mixture of **5.43** and **5.44** (8:2 by ^1H NMR). Recrystallization from ethyl acetate-

petroleum ether gave **5.43** (1 mg); [Found: $(M - Me)^+$, 312.1234. $C_{18}H_{18}NO_4$ requires *m/z* 312.1236]; mp 111-113 °C; $[\alpha]_D^{23}$ +24 (c 0.007 in CH_2Cl_2); NMR data identical to enantiomer **5.35**; *m/z* (EI) 312 (M^+ - Me, 0.1), 296 (M^+ - OMe, 2), 252 (43), 121 (100). The final fraction (2 mg) contained **5.43**.

(*1'R,2R*)- and (*1'S,2R*)-2-Hydroxy-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.45 and 5.46



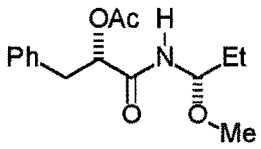
5.45



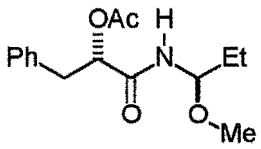
5.46

The acetates **5.43** (1.3 mg, 0.004 mmol) and **5.44** (2.3 mg, 0.007 mmol) were hydrolyzed (as described for **5.35** and **5.36** in the preparation of **5.18** and **5.19**) to give **5.45** (0.7 mg, 60%) and **5.46** (1.7 mg, 84%), respectively. **5.45** [Found: $(M - Me)^+$, 270.1128. $C_{16}H_{16}NO_3$ requires *m/z* 270.1130]; mp 99-101 °C; $[\alpha]_D^{23}$ +76 (c 0.004 in CH_2Cl_2); NMR data identical to enantiomer **5.18**; *m/z* (EI) 270 (M^+ - Me, 29), 253 (M^+ - MeOH, 33), 162 (39), 121 (100). **5.46** [Found: $(M - Me)^+$, 270.1134. $C_{16}H_{16}NO_3$ requires *m/z* 270.1130]; mp 62-64 °C; $[\alpha]_D^{23}$ +21 (c 0.009 in CH_2Cl_2); NMR data identical to enantiomer **5.19**; *m/z* (EI) 270 (M^+ - Me, 47), 253 (M^+ - MeOH, 43), 162 (49), 121 (100).

(*1'S,2S*)- and (*1'R,2S*)-2-Acetoxy-*N*-(1-methoxypropyl)-3-phenylpropanamide 5.47 and 5.48



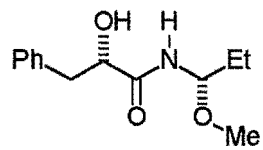
5.47



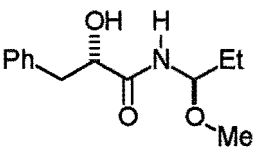
5.48

The amide **5.33** (0.25 mmol), prepared as described in the preparation of **5.35** and **5.36**, was reacted with **5.37** according to the general method B to give a mixture of **5.47** and **5.48** (49 mg, 70%). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:5 to 1:3) gave a mixture (12 mg) of **5.47** and **5.48** (1:1 by ^{1}H NMR) which were unable to be separated; ^{1}H NMR, **5.47/5.48** δ 0.78/0.83 (3H, t, CH_2CH_3), 1.42/1.57 (2H, m, CH_2Me), 2.09/2.11 (3H, s, Ac), 3.14/3.24 (3H, s, OMe), 3.15-3.22 (2H, m, CH_2Ph), 4.99 (1H, m, NCH), 5.35/5.40 (1H, m, CHCO), 6.10 (1H, br m, NH), 7.17-7.31 (5H, m, ArH); ^{13}C NMR, **5.47/5.48** δ 8.78 (CH_2Me), 20.67/20.85 (Ac), 28.28/28.53 (CH_2Me), 37.46/37.56 (CH_2Ph), 55.76/55.90 (OMe), 74.29/74.44 (CHCO), 82.27 (NCH), 126.97, 128.36, 128.39, 129.58 & 129.62 (ArCH), 135.57 & 135.66 (ArCR), 169.26 & 169.42 (CO).

(*1'S,2S*)- and (*1'R,2S*)- 2-Hydroxy-*N*-(1-methoxypropyl)-3-phenylpropanamide 5.49 and 5.50



5.49

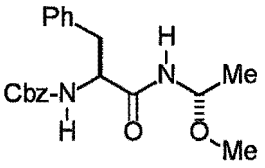


5.50

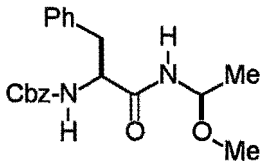
The mixture of **5.47** and **5.48** prepared by the general method B above (12 mg of a 1:1 mixture by ^{1}H NMR) was hydrolyzed in methanol and water (2.5 cm^3 of a 9:1 mixture) with potassium carbonate (0.2 equiv) at rt for 2 h. The mixture was

evaporated and redissolved in dichloromethane (2 cm^3). The organic solution was washed with water (2 cm^3), dried and evaporated to give an oil (11 mg, 92%) which was chromatographed on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (3:10) to give two fractions. The first fraction gave **5.49** as an oil (1 mg); [Found: $(M - Me)^+$, 222.1125. $C_{12}H_{16}NO_3$ requires *m/z* 222.1130]; $[\alpha]_D^{23}$ +104 (c 0.002 in CH_2Cl_2); IR /cm^{-1} 3400, 2972, 1680, 1301, 1085; ^1H NMR ($CDCl_3$, d_5-pyridine), δ 0.91 (3H, t, *J* 7.3, CH_2CH_3), 1.57 (1H, m, CH_2Me), 1.65 (1H, m, CH_2Me), 2.95 (1H, dd, *J* 13.7 & 8.3, CH_2Ph), 3.22 (3H, s, OMe), 3.28 (1H, m, CH_2Ph), 4.38 (1H, dd, *J* 8.3 & 3.9, CHCO), 5.03 (1H, m, NCH), 6.98 (1H, d, *J* 9.3, NH), 7.21-7.30 (5H, m, ArH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 8.96 (CH_2Me), 28.55 (CH_2Me), 41.05 (CH_2Ph), 55.56 (OMe), 72.70 (CHCO), 81.82 (NCH), 126.66, 128.45 & 129.65 (ArCH), 137.52 (ArCR); *m/z* (EI) 222 (M^+ - Me, 2), 208 (M^+ - C_2H_5, 13), 205 (M^+ - MeOH, 64), 91 (63), 73 (100). The second fraction gave **5.50** as an oil (1 mg); [Found: $(M - Me)^+$, 222.1085. $C_{12}H_{16}NO_3$ requires *m/z* 222.1130]; $[\alpha]_D^{23}$ +423 (c 0.002 in CH_2Cl_2); IR /cm^{-1} 3400, 2972, 1680, 1301, 1085; ^1H NMR ($CDCl_3$, d_5-pyridine), δ 0.84 (3H, t, *J* 7.3, CH_2CH_3), 1.48 (1H, m, CH_2Me), 1.65 (1H, m, CH_2Me), 2.91 (1H, dd, *J* 13.7 & 8.3, CH_2Ph), 3.28 (1H, dd, *J* 13.7 & 3.4, CH_2Ph), 3.31 (3H, s, OMe), 4.46 (1H, dd, *J* 8.3 & 3.4, CHCO), 5.03 (1H, m, NCH), 6.95 (1H, br s, NH), 7.18-7.33 (5H, m, ArH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 8.78 (CH_2Me), 28.20 (CH_2Me), 40.93 (CH_2Ph), 55.54 (OMe), 72.30 (CHCO), 81.70 (NCH), 126.22, 128.07 & 129.58 (ArCH), 137.88 (ArCR), 174.25 (CONH); *m/z* (EI) 222 (M^+ - Me, 1), 208 (M^+ - C_2H_5, 4), 205 (M^+ - MeOH, 27), 91 (36), 73 (100).

(*1'S,2S*)- and (*1'R,2S*)-2-(*N*-Benzyloxycarbonylamino)-*N*-(1-methoxyethyl)-3-phenylpropanamide 5.51 and 5.52



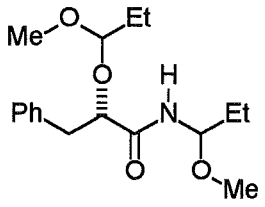
5.51



5.52

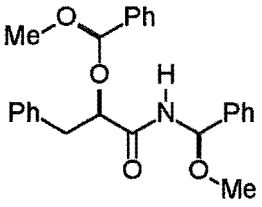
The amide **5.26** (61 mg, 0.21 mmol) was reacted with **5.38** according to the general method B to give a mixture of **5.51** and **5.52** (38 mg, 52%, 1:1 by ^{1}H NMR). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:5 to 1:1) gave three fractions. The first fraction (18 mg) gave **5.52** as a solid; [Found: $(M - OMe)^+$, 324.1474. $C_{19}H_{20}N_2O_3$ requires *m/z* 324.1474]; mp 136-138 °C; $[\alpha]_D^{23}$ -10 (c 0.007 in CH_2Cl_2); IR /cm^{-1} 3416, 3034, 1757, 1713, 1690, 1497; ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ 1.12 (3H, d, *J* 5.8, CH*Me*), 3.08 (2H, d, *J* 7.4, CHC*H*$_2$), 3.24 (3H, s, OMe), 4.47 (1H, m, C*H*CH$_2$), 5.08 (2H, s, CbzCH$_2$), 5.18 (1H, m, NCHO), 6.24 (1H, d, *J* 7.4, CbzNH), 7.11-7.36 (10H, m, ArH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 20.87 (CH*Me*), 38.44 (CHCH$_2$), 55.14 (OMe), 56.28 (*C*HCH$_2$), 66.53 (CbzCH$_2$), 77.51 (NCHO), 126.63, 127.62, 127.79, 128.18, 128.28 & 129.05 (ArC), 171.12 (CO); *m/z* (EI) 324 (M^+ - OMe, 2), 282 (M^+ - C_3H_7NO, 1), 91 (100). The second fraction (10 mg) contained a mixture of **5.52** and **5.51** (3:2 by ^{1}H NMR). The final fraction gave **5.51** (19 mg) (<10% **5.52** by ^{1}H NMR); [Found: $(M - OMe)^+$, 324.1474. $C_{19}H_{20}N_2O_3$ requires *m/z* 324.1474]; mp 158-160 °C; $[\alpha]_D^{23}$ +3 (c 0.020 in CH_2Cl_2); IR /cm^{-1} 3416, 3034, 1757, 1713, 1690, 1499; ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ 1.23 (3H, d, *J* 6.8, CH*Me*), 3.06 (2H, d, *J* 6.8, CHC*H*$_2$), 3.12 (3H, s, OMe), 4.55 (1H, m, C*H*CH$_2$), 5.06 (2H, s, CbzCH$_2$), 5.20 (1H, m, NCHO), 6.24 (1H, d, *J* 7.8, CbzNH), 7.16-7.30 (10H, m, ArH), 7.39 (1H, d, *J* 8.7, CONH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 20.99 (CH*Me*), 38.22 (CHCH$_2$), 55.08 (OMe), 56.20 (*C*HCH$_2$), 66.55 (CbzCH$_2$), 77.75 (NCO), 126.59, 127.62, 127.79, 128.17, 128.24 & 129.05 (ArCH), 155.84 (CbzCO), 166.12 (CONH); *m/z* (EI) 324 (M^+- OMe, 4), 282 (M^+ - C_3H_7NO, 1), 91 (100).

Reaction of the α-hydroxy primary amides 5.27 and 5.41 with the α-chloro ethers 5.37 and 5.34

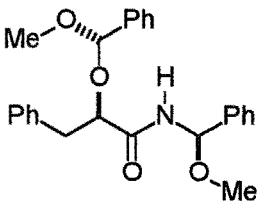


5.53

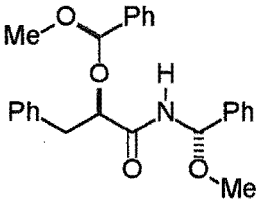
Amide **5.27** (9 mg, 0.06 mmol) was reacted directed with 3 equivalents of **5.37** and 5 equivalents of triethylamine in dichloromethane (1 cm^3) at rt for 18 h. The mixture was washed with water (2 cm^3), dried and evaporated to give a crude residue, which was purified by silica-based flash chromatography. Elution with ethyl acetate-dichloromethane (1:20 to 1:0) gave four fractions. The first fraction contained an mixture of four epimers, identified by ^{1}H NMR as having structure **5.53** (5 mg, 24%); ^{1}H NMR, δ 0.75-0.93 (6H, 8 t, CH_2*Me*), 1.36-1.70 (4H, m, C*H*$_2$Me), 2.87-3.23 (2H, m, CH_2Ph), 2.97, 3.04, 3.08, 3.12, 3.22, 3.24, 3.29 & 3.33 (6H total, 8 s, OMe), 4.10 & 4.14 (½H total, 2 t, *J* 5.3, OCHO), 4.24-4.37 (1½H, m, OCHO & C*H*CH_2), 4.96-5.01 (1H, m, NCH), 6.42, 6.52, 6.72 & 6.85 (1H total, 4 d, *J* 9.3-9.6, NH), 7.19-7.32 (5H, m, ArH). The second fraction contained **5.49** (2 mg, 18%). The third fraction contained a mixture of **5.50** and **5.49** (3:1 by ^{1}H NMR, 3 mg, 25%). The final fraction gave starting amide **5.27** (2 mg, 22%), which was formed by acid-catalyzed decomposition on silica.



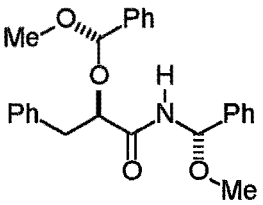
5.54



5.55



5.56

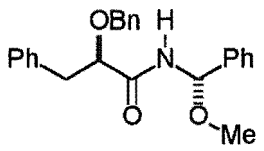


5.57

In a similar reaction, amide **5.41** was reacted with ether **5.34** according to the general method B to give an epimeric mixture of **5.54-5.57**. The mixture was purified twice on a 1 mm silica chromatatron plate, eluting with ethyl acetate-dichloromethane (0 : 1 to 1 : 1) to give fractions containing varying mixtures of **epimers A-D** (181 mg, 79%), corresponding in no particular order to **5.54-5.57**, as the configurations were unable to be assigned. The most pure fractions of epimers A-D are described as follows. A pure fraction of **epimer A** eluted first (4 mg), ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ 3.02 (1H, m, CH_2Ph), 3.01 (3H, s, OCHO*Me*), 3.31 (3H, s, NCHO*Me*), 3.34 (1H, dd, *J* 14.1 & 3.4, CH_2Ph), 4.44 (1H, dd, *J* 8.3 & 3.5, C*H*CH_2), 5.02 (1H, s, OCHO), 6.12 (1H, d, *J* 9.8, NCH), 7.10-7.46 (15H, m, ArH); ^{13}C NMR ($CDCl_3$, d_5-pyridine), δ 38.77 (CH_2Ph), 53.63 & 55.30 (OMe), 79.24 ($CHCH_2$), 80.82 (NCH), 104.76 (OCHO), 125.55, 126.17, 126.31, 127.95, 128.00, 128.07, 128.62, 129.51 (ArCH), 136.80, 136.97, 139.06 (ArCR), 171.71 (CO). A fraction containing a mixture of **epimer B**, epimer A and epimer C eluted second (4:2:1 by ^{1}H NMR, 18 mg), ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ epimer B (from the mixture) 2.93 (3H, s, OCHO*Me*), 3.07 (1H, dd, *J* 13.7 & 7.4, CH_2Ph), 3.25 (3H, s, NCHO*Me*), 3.21 (1H, dd, CH_2Ph), 4.48 (1H, dd, *J* 7.5 & 4.1, C*H*CH_2), 5.25 (1H, s, OCHO), 6.04 (1H, d, *J* 9.8, NCH), 7.08-7.33 (15H, m, ArH). A fraction containing a mixture of **epimer C**, epimer D and epimer B eluted third (11:6:1 by ^{1}H NMR, 47 mg), ^{1}H NMR ($CDCl_3$), δ epimer C (from the mixture) 3.01 (1H, m, CH_2Ph), 3.22 (3H, s, OCHO*Me*), 3.27 (1H,

m, CH_2Ph), 3.44 (3H, s, NCHO*Me*), 4.54 (1H, dd, *J* 7.8 & 4.0, C*H*CH_2), 5.18 (1H, s, OCHO), 6.11 (1H, d, *J* 9.8, NCH), 6.90 (1H, d, NH), 7.16-7.39 (15H, m, ArH). A pure fraction of **epimer D** eluted last (13 mg), ^{1}H NMR, δ 3.05 (2H, m, CH_2Ph), 3.08 (3H, s, OCHO*Me*), 3.32 (3H, s, NCHO*Me*), 4.56 (1H, dd, *J* 6.3 & 4.4, C*H*CH_2), 5.44 (1H, s, OCHO), 6.01 (1H, d, *J* 9.8, NCH), 6.89 (1H, d, *J* 9.7, NH), 7.05 (2H, m, ArH), 7.20-7.40 (13H, m, ArH); ^{13}C NMR, δ 39.19 (CH_2Ph), 53.40 & 55.89 (OMe), 76.33 ($CHCH_2$), 80.76 (NCH), 103.21 (OCHO), 125.64, 126.56, 126.61, 128.22, 128.35, 128.52, 129.10 & 129.91 (ArCH), 136.59, 137.08 & 138.67 (ArCR), 171.94 (CO).

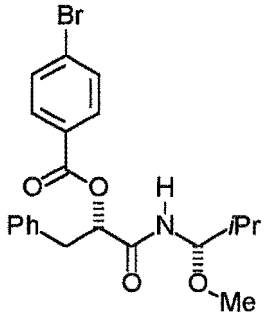
Derivatization of Analogue 5.46



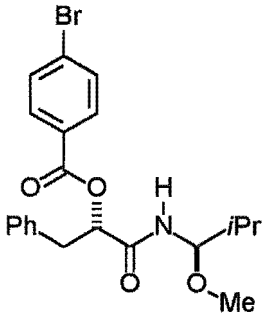
5.58

Compound **5.46** (8 mg, 0.03 mmol) was dissolved in dichloromethane (2 cm^3), and triethylamine (7 μL, 0.06 mmol), benzoyl chloride (6 μL, 0.06 mmol) and 4-dimethylaminopyridine (6 mg, 0.045 mmol) were added, and the mixture was stirred at rt for 18 h. The solution was washed with saturated aqueous $NaHCO_3$ (1 cm^3), dried and evaporated to give crude benzoate **5.58**, ^{1}H NMR, δ 3.36 (2H, m, CH_2Ph), 3.42 (3H, s, OMe), 5.75 (1H, t, *J* 5.9, C*H*CH_2), 6.14 (1H, d, *J* 9.3, NCH), 6.50 (1H, d, *J* 9.3, NH), 7.26 (10H, m, ArH), 7.45 (1H, m, ArH), 7.58 (2H, m, ArH), 7.97 (2H, m, ArH). Attempts at recrystallization of crude **5.58**, from diethyl ether-petroleum ether, gave crystals of benzoic acid/4-dimethylaminopyridine, suitable for X-ray crystallography (see chapter seven for discussion).

(*1'S,2S*)- and (*1'R,2S*)-2-(4-Bromobenzoyloxy)-*N*-(1-methoxy-2-methylpropyl)-3-phenylpropanamide 5.60 and 5.61



5.60

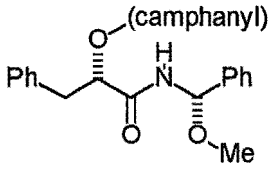


5.61

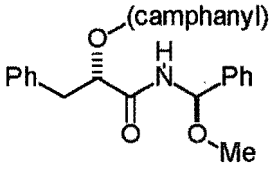
To a solution of amide **5.27** (55 mg, 0.33 mmol) and 4-dimethylaminopyridine (61 mg, 0.50 mmol) in dichloromethane (2.5 cm^3) was added triethylamine (92 μL, 0.66 mmol) and 4-bromobenzoyl chloride (81 mg, 0.57 mmol). After stirring the mixture at rt for 3 h the solvent was evaporated and benzene (5 cm^3) was added. The organic layer was washed with 2M aqueous HCl (3 cm^3), saturated aqueous $NaHCO_3$ (3 cm^3), water (3 cm^3) and dried. Evaporation under reduced pressure gave **5.59** (117 mg) which was used without further purification; ^1H NMR ($CDCl_3$), δ 3.31 (2H, m, CH_2Ph), 5.60 (1H, m, CHO), 5.77 (1H, br s, NH), 6.00 (1H, br s, NH), 7.23-7.29 (5H, m, ArH), 7.59 (2H, m, ArH), 7.82 (2H, m, ArH). The amide **5.59** was reacted with **5.39** according to the general method B to give a crude mixture of **5.60** and **5.61** (150 mg). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (3:50) gave a mixture of **5.60** and **5.61** (17 mg, 12% yield from **5.27**, 1:1 by ^1H NMR). The mixture was further purified on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:50) to give four fractions. The first fraction (1 mg) gave **5.60** (<5% **5.61** by ^1H NMR) as an oil; [Found: $(M - MeOH)^+$, 403.0609 & 401.0635. $C_{20}H_{20}NO_3Br$ requires *m/z* 403.0606 & 401.0627]; ^1H NMR, δ **5.60** (from the mixture) 0.75 (3H, d, *J* 6.9, CH*Me*), 0.81 (3H, d, *J* 6.4, CH*Me*), 1.68 (1H, m, C*H*Me_2), 3.16 (3H, s, OMe), 3.34 (2H, m, CH_2Ph), 4.83 (1H, dd, *J* 5.8 & 9.7, NCH), 5.60 (1H, t, *J* 5.9, C*H*CH_2), 5.93 (1H, d, *J* 9.3, NH), 7.22-7.28 (5H, m, ArH), 7.62 (2H, m, ArH), 7.86 (2H, m, ArH); *m/z* (EI) 403 [$M^+(^{81}Br)$ - MeOH, 3], 401 [$M^+(^{79}Br)$ - MeOH, 4], 333 (9), 185 (95), 183 (100). The second fraction (7 mg)

contained a mixture of **5.60** and **5.61** (2:1 by ^{1}H NMR). The third fraction (6 mg) contained a mixture of **5.61** to **5.60** (2:1 by ^{1}H NMR). The final fraction (3 mg) contained a mixture of **5.61** to **5.60** (17:3 by ^{1}H NMR); [Found: $(M - MeOH)^+$, 403.0608 & 401.0633. $C_{20}H_{20}NO_3Br$ requires *m/z* 403.0606 & 401.0627]; ^{1}H NMR, δ **5.61** (from the mixture) 0.76 (3H, d, *J* 6.9, CH*Me*), 0.79 (3H, d, *J* 6.4, CH*Me*), 1.68 (1H, m, C*H*Me$_2$), 3.22 (3H, s, OMe), 3.33 (2H, m, CH_2Ph), 4.83 (1H, dd, *J* 5.8 & 9.7, NCH), 5.64 (1H, t, *J* 5.9, C*H*CH_2), 5.97 (1H, d, *J* 9.3, NH), 7.22-7.27 (5H, m, ArH), 7.62 (2H, m, ArH), 7.84 (2H, m, ArH); *m/z* (EI) 403 [M^+(^{81}Br) - MeOH, 3], 401 [M^+(^{79}Br) - MeOH, 3], 333 (8), 185 (87), 183 (85).

(*1'S,2S*)- and (*1'R,2S*)-2-[(*1S,4R*)-Camphanyloxy]-*N*-(1-methoxybenzyl)-3-phenylpropanamide 5.63 and 5.64



5.63



5.64

A solution of (*1S,4R*)-camphanic acid (66 mg, 0.33 mmol) in thionyl chloride (5 cm^3) was refluxed for 2 h. Evaporation of the solvent under reduced pressure gave an oil. The oil was dissolved in dichloromethane (1 cm^3) and the solution added to a stirred solution of **5.27** (24 mg, 0.15 mmol), 4-dimethylaminopyridine (18 mg, 0.15 mmol) and diisopropylethylamine (28 μL, 0.16 mmol) in dichloromethane (1 cm^3). After stirring for 18 h at rt the reaction mixture was washed with water (2 cm^3) and dried. Evaporation under reduced pressure gave **5.62** (65 mg) which was used without further purification; ^{1}H NMR ($CDCl_3$), δ 0.71 (3H, s, camphanyl-Me), 0.96 (3H, s, camphanyl-Me), 0.98 (3H, s, camphanyl-Me), 5.57 (1H, m, C*H*CH_2Ph), 6.11 (1H, br s, NH), 6.61 (1H, br s, NH). The amide **5.62**, (65 mg, 0.19 mmol) was reacted with **5.34** according to the general method B to give a mixture of **5.63** and **5.64** (44 mg, 51%). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (2:3) gave a mixture of **5.63** to **5.64** (26 mg) (1:1 by ^{1}H NMR).

Further purification on a 1 mm chromatatron plate eluting with ethyl acetate-petroleum ether (1:4 to 3:7) gave three fractions. The first fraction (14 mg) contained a mixture of **5.64** and **5.63** (3:2 by ^{1}H NMR); [Found: (M - OMe)$^+$, 434.1942. $C_{27}H_{31}NO_6$ requires *m/z* 434.1968]; ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ **5.64** (from the mixture) 0.71 (3H, s, camphanyl-Me), 0.97 (3H, s, camphanyl-Me), 1.07 (3H, s, camphanyl-Me), 1.65 (1H, m, CH_2CH_2), 1.90 (2H, m, CH_2CH_2), 2.33 (1H, m, CH_2CH_2), 3.16 (1H, dd, *J* 14.6 & 8.3, CH_2Ph), 3.33 (1H, dd, *J* 14.6 & 4.6, CH_2Ph), 3.39 (3H, s, OMe), 5.52 (1H, dd, *J* 8.3 & 4.6, C*H*CH_2), 6.10 (1H, d, *J* 9.3, NCH), 7.19-7.37 (10H, m, ArH); m/z (EI) 434 (M$^+$ - OMe, 11), 329 (5), 273 (82), 131 (100). The second fraction (9 mg) contained a mixture of **5.63** and **5.64** (7:3 by ^{1}H NMR). Recrystallization from ethyl acetate-petroleum ether gave crystals of **5.63** suitable for X-ray crystallography. The final fraction (2 mg) gave **5.63** (<10% **5.64** by ^{1}H NMR); [Found: (M - OMe)$^+$, 434.1974. $C_{27}H_{31}NO_6$ requires *m/z* 434.1968]; ^{1}H NMR ($CDCl_3$, d_5-pyridine), δ 0.76 (3H, s, camphanyl-Me), 1.00 (3H, s, camphanyl-Me), 1.08 (3H, s, camphanyl-Me), 1.60 (1H, m, CH_2CH_2), 1.76 (1H, m, CH_2CH_2), 1.87 (1H, m, CH_2CH_2), 2.26 (1H, m, CH_2CH_2), 3.17 (1H, dd, *J* 14.4 & 8.5, CH_2Ph), 3.30 (1H, dd, *J* 14.2 & 5.4, CH_2Ph), 3.42 (3H, s, OMe), 5.41 (1H, dd, *J* 8.4 & 5.4, C*H*CH_2), 6.11 (1H, d, *J* 9.3, NCH), 7.17-7.32 (10H, m, ArH), 7.99 (1H, d, *J* 9.3, NH); *m/z* (EI) 434 (M$^+$ - OMe, 6), 330 (7), 273 (70), 131 (100).

A mixture (5 mg, 72%) of **5.64** and **5.63** (9:1 by ^{1}H NMR) was also prepared from a mixture (5mg, 0.015 mmol) of **5.19** and **5.18** (9:1 by ^{1}H NMR) by an identical procedure to the above preparation of camphanyl ester **5.62** from amide **5.27**.

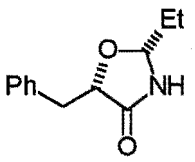
X-ray Crystallographic Determination for Compound 5.63

$C_{27}H_{31}NO_6$, *M* = 465.53, crystal dimensions 0.7 x 0.2 x 0.08, Triclinic, a = 6.2953(13), b = 12.667(3), c = 15.522(3) Å, α = 88.73(3), β = 86.45(3), γ = 85.19(3)°, *V* = 1230.9(4) Å^3, spacegroup P1, *Z* = 2, F(000) 496, Dcalc 1.256 Mg/m^3, μ(Cu-Kα) = 6.71 cm^{-1}, absorption coefficient 0.722 mm^{-1}, θ range for data collection 2.85 to 60.10, index ranges $0 \le h \le 7$, $-14 \le k \le 14$, $-17 \le l \le 17$, minimum and maximum transmissions 0.8829 and 1.0000, data/restraints/parameters 4053 / 3 / 621, goodness of fit on F^2 was 1.121, final R indices [I>2s(I)] R_1 = 0.0282, wR_2 = 0.0739,

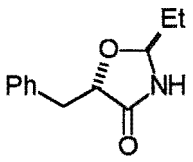
R indices (all data) $R_1 = 0.0408$, $wR_2 = 0.1017$, largest difference peak and hold 0.146 and -0.142eÅ^{-3}.

The unit cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections with $106.4^\circ \leq 2\theta \leq 114.7^\circ$ from a Rigaku diffractometer. A unique data set was measured at 293(2)K within $2\theta_{max} = 120.2^\circ$ limit (ω-2θ). Of the 4056 reflections obtained, 3665 were unique ($R_{int} = 0.024$) and were used in the full-matrix least-squares refinement[154] after being corrected for absorption by using the psi-scan method. The intensities of 3 standard reflections, measured every 150 reflections throughout the data collection, showed only 1.27% decay. The structure was solved by direct methods.[155] Hydrogen atoms were fixed in idealized positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.[156] The functions minimised were $\Sigma w(F_o^2 - F_c^2)$, with $w = [\sigma^2(F_o^2) + 0.045P^2 + 0.044P]^{-1}$ where $P = [\max(F_o)^2 + 2F_c^2]/3$. Full tables of atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Preparation of *cis*-(*2R,5S*)- and *trans*-(*2S,5S*)-5-benzyl-2-ethyloxazolidin-4-one 5.65 and 5.66



5.65

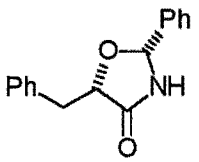


5.66

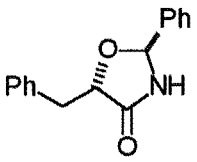
An excess of **5.37** (5 equiv) was added to the amide **5.27** (124 mg, 0.73 mmol) in dichloromethane (5 cm^3) and the mixture was stirred at rt for 18 h. The solution was washed with 10% aqueous $NaHCO_3$ (5 cm^3), water (5 cm^3), dried and evaporated under reduced pressure to give a crude mixture of **5.66** and **5.65** (208 mg, 13:7 by ^{1}H NMR). Purification of the crude mixture on a 1 mm silica chromatatron plate eluting with ethyl acetate-dichloromethane (0:1 to 3:10) gave three fractions. The first

fraction (6 mg) contained **5.66** as an oil; (Found: M^+, 205.1103. $C_{12}H_{15}NO_2$ requires *m/z* 205.1103); ^{1}H NMR, δ 0.90 (3H, t, *J* 7.5, Me), 1.60 (2H, m, C*H*$_2$Me), 3.05 (2H, m, CH_2Ph), 4.57 (1H, m, C*H*CH_2), 4.85 (1H, m, NCH), 6.46 (1H, br s, NH), 7.29 (5H, m, ArH); ^{13}C NMR, δ 7.17 (Me), 29.50 (*C*H_2Me), 37.83 (CH_2Ph), 77.91 (*C*HCH_2), 87.67 (NCH), 126.74, 128.33 & 129.80 (ArCH), 136.50 (ArCR), 175.99 (CO); *m/z* (EI) 205 (M^+, 54), 176 (M^+ - C_2H_5, 49), 131 (M^+ - C_3H_8NO, 87), 91 (100). The second fraction (6 mg) contained a mixture of **5.66** to **5.65** (4:1 by ^{1}H NMR). The final fraction (13 mg) gave **5.65** as an oil; (Found: M^+, 205.1106. $C_{12}H_{15}NO_2$ requires *m/z* 205.1103); ^{1}H NMR, δ 0.87 (3H, t, *J* 7.6, Me), 1.46 (2H, m, C*H*$_2$Me), 3.07 (2H, m, CH_2Ph), 4.49 (1H, m, C*H*CH_2), 5.10 (1H, m, NCH), 6.88 (1H, br s, NH), 7.28 (5H, m, ArH); ^{13}C NMR, δ 7.31 (Me), 29.05 (*C*H_2Me), 37.89 (CH_2Ph), 78.20 (*C*HCH_2), 87.17 (NCH), 126.65, 128.22 & 129.72 (ArCH), 136.87 (ArCR), 174.09 (CO); *m/z* (EI) 205 (M^+, 40), 176 (M^+ - C_2H_5, 78), 131 (M^+ - C_3H_8NO, 73), 91 (100).

Preparation of *cis*-(*2R,5S*)- and *trans*-(*2S,5S*)-5-benzyl-2-phenyloxazolidin-4-one 5.67 and 5.68



5.67

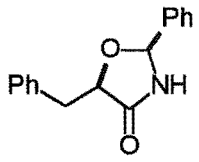


5.68

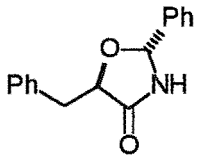
An excess of **5.34** (25 equiv) was added to the amide **5.27** (93 mg, 0.56 mmol) in dichloromethane (5 cm^3) and the mixture was left to stand at -10 °C for 18 h. The solution was washed with 10% aqueous $NaHCO_3$ (5 cm^3), water (5 cm^3), dried and evaporated under reduced pressure to give a crude mixture of **5.67** and **5.68** (141 mg). Purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (2:3) gave a mixture (76 mg, 54%) of **5.68** and **5.67** (2:1 by ^{1}H NMR). Further purification on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:9 to 1:0) gave two fractions. The first fraction gave **5.68** as an oil (19 mg);

(Found: M^+, 253.1102. $C_{16}H_{15}NO_2$ requires *m/z* 253.1103); $[\alpha]_D^{23}$ -17 (c 0.007 in CH_2Cl_2); IR /cm^{-1} 3429, 1756, 1728, 1278, 1247, 1126; ^1H NMR, δ 3.13 (2H, m, CH_2Ph), 4.77 (1H, m, C*H*CH_2), 5.72 (1H, d, *J* 2.4, NCH), 7.24-7.37 (10H, m, ArH), 7.86 (1H, br s, NH); ^{13}C NMR, δ 37.57 (CH_2Ph), 78.19 (*C*HCH_2), 87.81 (NCH), 126.22, 126.79, 128.31, 128.69, 129.66 & 129.73 (ArCH), 136.09 & 138.28 (ArCR), 166.36 (CO); *m/z* (EI) 253 (M^+, 76), 106 (100). The second fraction gave **5.67** as an oil (11 mg) ; (Found: M^+, 253.1105. $C_{16}H_{15}NO_2$ requires *m/z* 253.1103); $[\alpha]_D^{23}$ -121 (c 0.007 in CH_2Cl_2); IR /cm^{-1} 3430, 1730, 1498, 1462, 1313, 1081; ^1H NMR, δ 3.11 (2H, m, CH_2Ph), 4.59 (1H, m, C*H*CH_2), 5.96 (1H, d, *J* 2.1, NCH), 7.03-7.07 (2H, m, ArH), 7.18-7.36 (8H, m, ArH), 7.75 (1H, br s, NH); ^{13}C NMR, δ 37.42 (CH_2Ph), 78.51 (*C*HCH_2), 87.12 (NCH), 126.61, 126.99, 128.29, 128.51, 129.81 & 129.92 (ArCH), 136.45 & 137.50 (ArCR), 174.47 (CO); *m/z* (EI) 253 (M^+, 25), 147 (90), 106 (100).

Preparation of *cis*-(*2S,5R*)- and *trans*-(*2R,5R*)-5-benzyl-2-phenyloxazolidin-4-ones 5.69 and 5.70



5.69

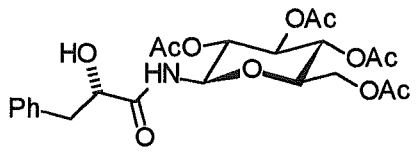


5.70

An excess of **5.34** (25 equiv) was added to the amide **5.41** (40 mg, 0.24 mmol) in dichloromethane (2 cm^3) and the mixture was stirred at rt for 18 h. Workup as above gave a crude mixture of **5.70** and **5.69** (141 mg, 3:2 by ^1H NMR), which was purified by flash chromatography, eluting with ethyl acetate-dichloromethane (1:20 to 1:5) to give two fractions. The first fraction (16 mg) contained a mixture of **5.70** and **5.69** (7:3 by ^1H NMR). The second fraction gave **5.69** as an oil (16 mg); (Found: M^+, 253.1105. $C_{16}H_{15}NO_2$ requires *m/z* 253.1103); ^1H NMR data identical to enantiomer **5.67**. Fraction 1 was further purified on a 1 mm silica chromatatron plate eluting with ethyl acetate-petroleum ether (1:3 to 1:1) to give **5.70** (2 mg) as an oil; (Found: M^+,

253.1102. $C_{16}H_{15}NO_2$ requires *m/z* 253.1103); ^{1}H NMR data identical to enantiomer **5.68**. Further elution gave more **5.69** (3 mg).

Preparation of (*2S*)-2-hydroxyl-3-phenyl-*N*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)propanamide 5.76



5.76

To a stirred mixture of *D*-glucose **5.71** (5.020 g, 27.80 mmol) and acetic anhydride (21.8 cm^3, 231 mmol) was added conc. sulphuric acid (3 drops). The solid dissolved over 10 min and the temperature of the mixture rose to approximately 100 °C. The solution was then heated on a steam bath for 2 h. The remaining acetic acid and acetic anhydride were removed by vacuum distillation (water pump) to give a viscous light yellow syrup of pentaacetate **5.72**. A solution of 40% HBr in acetic acid (10 cm^3) was added to the syrup at 0 °C and the solution was stirred at rt for 3 h. Evaporation under reduced pressure and crystallisation from diisopropyl ether gave *2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide*[141] **5.73** (4.690 g, 94%); mp 87-88 °C (literature mp 87-88 °C); ^{1}H NMR, δ 2.04 (3H, s, Ac), 2.06 (3H, s, Ac), 2.10 (3H, s, Ac), 2.11 (3H, s, Ac), 4.13 (1H, m), 4.33 (2H, m), 4.86 (1H, dd, *J* 10.0 & 4.1), 5.18 (1H, t, *J* 10.0), 5.55 (1H, t, *J* 9.8), 6.62 (1H, d, *J* 3.9, CHBr); ^{13}C NMR, δ 20.06, 20.11, 20.14, 60.49, 66.65, 69.69, 70.04, 71.77, 86.44, 168.93, 169.20, 169.27, 169.89.

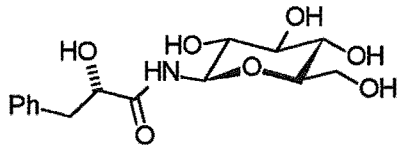
A mixture of **5.73** (1.842 g, 4.50 mmol) and sodium azide (0.817 g, 12.60 mmol) in formamide (15 cm^3) was stirred at 80-85 °C for 3 h. After cooling, the mixture was poured into water (50 cm^3) and extracted with chloroform (3 x 15 cm^3). The organic extracts were washed with water (10 cm^3), dried and evaporated to yield a dark oil, which crystallized after 18 h at -10 °C. Recrystallization from ethyl acetate-petroleum ether gave *2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide* **5.74** (355 mg); mp 126-127 °C (literature[142] mp 124-125 °C). The mother liquor was evaporated to yield less

pure **5.74** (854 mg, 72% combined yield); ^{1}H NMR, δ 2.02 (3H, s, Ac), 2.04 (3H, s, Ac), 2.09 (3H, s, Ac), 2.11 (3H, s, Ac), 3.80 (1H, m, glucH5), 4.17 (1H, m, glucH6a), 4.28 (1H, m, glucH6b), 4.65 (1H, d, *J* 8.8, glucH1), 4.96 & 5.11 & 5.23 (3H, 3 t, glucH2 & H3 & H4); ^{13}C NMR, δ 20.48, 20.64, 61.57, 67.78, 70.54, 72.52, 73.94, 87.83, 169.16, 169.27, 170.07, 170.58.

A solution of **5.74** (198 mg, 0.53 mmol) in ethyl acetate (20 cm^3) was catalytically hydrogenated over PtO_2 (19 mg, 0.085 mmol) at atmospheric pressure for 3.5 h. The mixture was filtered and evaporated to yield a white solid residue of *2,3,4,6-tetra-O-acetyl-β-D-glucosylamine* **5.75** (156 mg, 85%); mp 125-126 °C (literature[143] mp 126 °C); ^{1}H NMR, δ 1.94 (3H, s, Ac), 1.96 (3H, s, Ac), 1.97 (3H, s, Ac), 2.00 (3H, s, Ac), 2.90 (2H, br s, NH_2), 3.65 (1H, m, glucH5), 4.06 (1H, m, glucH6a), 4.16 (2H, m, glucH1 & H6b), 4.76 & 4.97 & 5.18 (3H, t, glucH2 & H3 & H4); ^{13}C NMR, δ 20.22, 20.36 & 20.40 (Ac), 61.97, 68.42, 71.73, 72.31, 72.84, 84.44 (CNH_2), 169.25, 169.81 & 170.39 (CO).

A mixture of the acid **5.15** (50 mg, 0.30 mmol), glucosylamine **5.75** (104 mg, 0.30 mmol), 1-hydroxybenzotriazole (42 mg, 0.30 mmol) and dicyclohexylcarbodiimide (61 mg, 0.30 mmol) in dichloromethane (10 cm^3) was stirred at rt for 5 d. The mixture was filtered and evaporated to yield crude amide **5.76** (218 mg). The amide was loaded onto a 1 mm chromatatron plate and eluted with ethyl acetate-petroleum ether (1:1) to give **5.76** (87 mg, 59%), which was recrystallized from ethyl acetate-petroleum ether to give fine white crystals of **5.76** (15 mg); (Found: MH$^+$, 496.1810. $C_{23}H_{30}NO_{11}$ requires *m/z* 496.1819); mp 172.5-174.5 °C; $[\alpha]_D^{23}$ +46 (c 0.007 in CH_2Cl_2); ^{1}H NMR, δ 1.94 (3H, s, Ac), 2.02 (3H, s, Ac), 2.03 (3H, s, Ac), 2.07 (3H, s, Ac), 2.78 (1H, dd, *J* 13.6 & 8.5, CH_2Ph), 2.86 (1H, d, *J* 4.8, OH), 3.18 (1H, dd, *J* 13.7 & 2.9, CH_2Ph), 3.82 (1H, m, glucH5), 4.11 (1H, m, glucH6a), 4.31 (1H, m, glucH6b), 4.33 (1H, m, C*H*CH_2Ph), 4.94 (1H, t, *J* 9.5, glucH2), 5.06 (1H, t, *J* 9.5, glucH4), 5.21 (1H, t, *J* 9.3, glucH1), 5.30 (1H, t, *J* 9.5, glucH3), 7.23-7.34 (4H, m, ArH), 7.46 (1H, m, ArH); ^{13}C NMR, δ 20.50 & 20.63 (Ac), 40.32 (CH_2Ph), 61.52 (glucC6), 67.97 (glucC4), 70.36 (glucC2), 72.59 (glucC3), 72.71 (*C*HCH_2Ph), 73.56 (glucC5), 77.82 (glucC1), 126.97, 128.62 & 129.52 (ArCH), 136.40 (ArCR), 169.47, 170.48 & 173.57 (CO); IR /cm^{-1} 3052, 1756, 1225, 1043; *m/z* (FAB) 496 (MH$^+$, 53), 169 (100).

Preparation of (*2S*)-*N*-(β-*D*-glucopyranosyl)-2-hydroxyl-3-phenylpropanamide 5.77



5.77

The amide **5.76** (37 mg, 0.075 mmol) and potassium carbonate (2 mg, 0.015 mmol) were dissolved in methanol-water (4 cm^3 of a 9:1 mixture) and the solution was stirred at rt for 1 h. The methanol was removed under reduced pressure and the aqueous layer was washed with dichloromethane (3 x 5 cm^3) and evaporated to give **5.77** (24 mg, 96%) ; (Found: MK^+, 366.0960. $C_{16}H_{21}NO_7K$ requires *m/z* 366.0955); ^1H NMR (D_2O), δ 1.77 (1H, s, OH), 2.84 (1H, dd, *J* 14.2 & 7.8, CH_2Ph), 3.02 (1H, dd, *J* 14.1 & 4.9, CH_2Ph), 3.25-3.77 (6H, m, glucH2-H6), 4.36 (1H, dd, *J* 7.8 & 4.9, C*H*CH_2Ph), 4.81 (1H, d, *J* 9.3, glucH1), 7.16-7.26 (5H, m, ArH); *m/z* (FAB) 366 (MK^+, 8), 307 (10), 154 (100).

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